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Phosphoenolpyruvate:Carbohydrate Phosphotransferase Systems of Bacteria

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INTRODUCTION

Microorganisms display an amazing capacity for metabolic versatility. Most bacteria can utilize a variety of carbon sources and adapt themselves to their continuously changing surroundings to be able to effectively compete with other organisms for limiting nutrients. Bacteria, like many other cells, contain sensing devices to monitor their surroundings. They can turn on and off the utilization of a large number of carbon sources, sense concentration gradients of nutrients accordingly, and adapt to changes in osmotic strength, to stress conditions, to oxygen (or the lack of it), and to limited nutrients. Many signals are sensed on the outside of the cell and are converted into a response which can involve a change in the synthesis of proteins, the regulation of enzyme activity, changes in behavior (e.g., motility), or other processes. Many such sensory systems have been identified, and one common theme connects most of these systems: phosphorylation of the proteins involved, on histidine, serine, and aspartate residues.

The topic of this review is one of these sensory systems, the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS). This system is involved in both the transport and phosphorylation of a large number of carbohydrates, in movement toward these carbon sources (chemotaxis), and in the regulation of a number of other metabolic pathways. In the nearly 30 years since its discovery in *Escherichia coli* (231), we have learned many details about the properties and the functioning of the many PTS proteins in both gram-negative and gram-positive microorganisms. Our appreciation of the complexity of this system has steadily increased: at present we know of at least 20 different PTS proteins and 10 non-PTS proteins that interact with various PTS proteins.

Eight years ago we published a review (358) that covered the literature on the PTS up to 1985. Since that time more than 400 papers on the PTS have been published. In particular, progress has been made in the characterization of the many carbohydrate-specific PTS proteins of both gram-negative and gram-positive bacteria as a result of the cloning and sequencing of many of the corresponding genes. The three-dimensional structures of a few of the soluble PTS proteins have also been determined.

The purpose of the present review is to give an overview of the PTS with particular emphasis on results published since 1985. References to the earlier literature are included if required to present a coherent picture. Although every effort has been made to provide a comprehensive review of the recent literature in particular, space limitations have made a certain amount of selectivity inevitable. Finally, the reader is also referred to a number of other recent reviews that deal with specific aspects of the PTS (380, 385, 396, 418, 420, 481), give a general overview (284, 353), present a historical perspective (407), or contain collections of general articles (59) or articles dealing with gram-positive organisms (381).

OVERVIEW AND PTS COMPONENTS

Overall Reaction Catalyzed by PTS, and Thermodynamic Considerations

Regardless of the organism or carbohydrate, all PTSs that have been characterized catalyze the following overall process:

$$\text{P-enolpyruvate}_{(in)} + \text{carbohydrate}_{(out)} \xrightarrow{PTS} \text{pyruvate}_{(in)} + \text{carbohydrate-P}_{(in)}$$

Carbohydrate phosphorylation is coupled to its translocation across the membrane, the energy for these processes being provided by the glycolytic intermediate PEP.

Although the free energy of hydrolysis ($\Delta G^{\circ\prime}$) of the phospho group of PEP is about −14.7 kcal/mol (−61.5 kJ/mol), that of a typical carbohydrate phosphate ester is ca. −3 kcal/mol (ca. −12.5 kJ/mol) (12). Therefore, at first glance this would seem to be a waste of energy. However, unlike any other well-characterized carbohydrate transport system, the PTS accomplishes both the translocation and phosphorylation of its substrates. Although the phosphoryl transfer potential of PEP is higher than that of ATP (12), PEP is nonetheless equivalent to ATP in the "energy currency" of the cell, since, during glycolysis, one ATP molecule is derived from one PEP molecule in the pyruvate kinase reaction. For carbohydrates that are actively accumulated by non-PTS systems, more than one ATP equivalent must be expended per monosaccharide unit for both transport and subsequent ATP-dependent phosphorylation. This is undoubtedly one reason why the PTS is found mainly in obligate and facultative anaerobic bacteria, which synthesize ATP by substrate-level phosphorylation under anaerobic

TABLE 1. Well-characterized EIs and HPrs of the PTS[a]

Protein and organism	M_r[b]	References[c]
Enzyme I		
E. coli	**63,489** (dimer)	394, 518 (P); 39, 72, 416 (S)
S. typhimurium	**63,412** (dimer)	525 (P); 39, 256, 443 (S)
S. carnosus	**63,369**	218 (S)
B. subtilis	66,000	388 (P); 142 (s)
M. capricolum[d]	44,500 (α_2)	192 (P)
	62,000 (β)	
	64,500 (γ)	
E. faecalis	70,000 (dimer)	5 (P and s)
S. aureus	85,000	166 (P); 4 (s)
S. salivarius	**62,948** (dimer)	496 (P); 129 (S)
S. mutans	68,000 (dimer)	483 (P)
HPr		
E. coli	**9,109**	9, 87 (P); 39, 72, 416, 524 (S)
S. typhimurium	**9,109**	16 (P); 39, 363, 443, 524 (S)
K. pneumoniae	**9,109**	490 (S)
B. subtilis	**9,121**	205, 277, 375, 387 (P); 142 (S)
M. capricolum	10,000	191 (P)
S. aureus	**9,357**[e]	20, 454 (P); 20, 385 (S)
S. carnosus	**9,500**[f]	91 (P); 91 (S)
E. faecalis	**9,438**	81, 205 (P); 81 (S)
S. salivarius	7,000–13,000[g]	496, 515 (P)
S. mutans	7,000–17,000[g]	291, 483 (P)
S. sanguis	16,000[g]	195 (P)
FPr[h]		
E. coli	36,000–40,000	513 (P); 134 (c)
S. typhimurium	**39,593**	477 (P); 133 (S)
MTP[i]		
R. capsulatus	**86,360**	542 (S)

[a] Proteins that have been purified to homogeneity and/or for which a complete or partial gene sequence is known.

[b] Numbers in boldface type are precise molecular weights calculated from the determined or deduced amino acid sequence. Other M_rs are estimates of the monomer molecular weight from denaturing gel electrophoresis. EIs for which there is good evidence that the native protein is a homodimer are indicated.

[c] Abbreviations: P, references for purification of the protein; S, references for the complete sequence of the gene or protein; s, references for only a partial sequence; c, molecular weight determined by the size of the product expressed from the cloned gene.

[d] The unusual EI from *M. capricolum* appears to consist of three different polypeptides in the stoichiometry indicated. However, further biochemical and genetic work is necessary to confirm whether these are distinct polypeptides and whether all three are necessary for EI activity.

[e] Calculated from the deduced amino acid sequence as revised (385) from reference 20.

[f] Calculated from the published amino acid sequence (91).

[g] These HPrs show extreme variability of apparent M_r depending on the gel system used. It is likely that the true molecular weight is closer to the lower value given (483).

[h] FPr is composed of an $EIIA^{Fru}$ domain (amino-terminal) and an HPr-like domain (carboxy-terminal). The molecular weight for the *E. coli* protein was estimated in a maxicell expression system (134).

[i] Multiphosphoryl transfer protein; MTP is composed of an $EIIA^{Fru}$ domain, an HPr-like domain, and an EI-like domain (from amino to carboxy terminus).

conditions and thus must make judicious use of the ATP so generated (for a recent compilation of bacteria for which the presence or absence of the PTS has been documented, see reference 402). In these organisms, the PTS also represents a self-priming transport system, since an intermediate in the catabolism of its substrates is also used for accumulation and phosphorylation of these substrates. Furthermore, at least some of the PTS proteins may phosphorylate other proteins in the cell in their roles in chemotaxis and metabolic regulation. The high phosphate transfer potential of PEP is largely retained in the phosphorylated derivatives of PTS proteins (523), and this may facilitate phosphotransfer from PTS proteins to protein components of these other systems.

Roles and Organization of PTS Proteins

The proteins that make up the PTS have been most extensively studied in the enteric bacteria *E. coli* and *Salmonella typhimurium*, although these proteins have been partially characterized in a variety of other bacteria (cf. Table 1 and Table 2). In all organisms studied, the following reactions compose the PTS-mediated translocation and phosphorylation of a given carbohydrate:

$$\text{P-enolpyruvate} + \text{enzyme I (EI)} \rightleftharpoons \text{P-EI} + \text{pyruvate} \quad (1)$$

$$\text{P-EI} + \text{HPr} \rightleftharpoons \text{P-HPr} + \text{EI} \quad (2)$$

$$\text{P-HPr} + \text{EIIA (domain or protein)} \rightleftharpoons \text{P-EIIA} + \text{HPr} \quad (3)$$

$$\text{P-EIIA} + \text{EIIB (domain or protein)} \rightleftharpoons \text{P-EIIB} + \text{EIIA} \quad (4)$$

$$\text{P-EIIB} + \text{carbohydrate}_{(out)} \xrightarrow{\text{EIIC}} \text{EIIB} + \text{carbohydrate-P}_{(in)} \quad (5)$$

In most cases, enzyme I (EI) and HPr are soluble, cytoplasmic proteins that participate in the phosphorylation of all PTS carbohydrates in a given organism and thus have been called the general PTS proteins. On the other hand, the enzymes II (EIIs) are carbohydrate specific and may consist of a single membrane-bound protein composed of three domains (A, B, and C), such as that for mannitol (EII^{Mtl}) in *E. coli*, or of two or more proteins, at least one of which is membrane bound (e.g., B and C) and one of which is soluble (IIA or enzyme III [EIII]) such as the $IICB^{Glc}$-IIA^{Glc} pair for glucose in *E. coli* (Fig. 1). In both cases, the phospho group is transferred from PEP to the incoming carbohydrate via obligatory phospho intermediates of EI, HPr, EIIA, and EIIB. The EIIC domain, which makes up the integral membrane portion of an EII, presumably forms its translocation channel and at least part of its specific substrate-binding site. In a third variation, exemplified by the mannose PTS in *E. coli*, both A and B domains are fused in a single soluble polypeptide, while there are two integral membrane proteins (IIC^{Man} and IID^{Man}) involved in mannose translocation (Fig. 1). Figure 1 illustrates schematically the phosphotransfer reactions of the PTS, as well as its vectorial nature with respect to transport and phosphorylation, for the *E. coli* mannitol, glucose, and mannose PTSs.

Although the glucose, mannitol, and mannose PTSs of *E. coli* are the most common in terms of protein organization in most organisms, other variations are possible. For example, the cellobiose PTS in *E. coli* has recently been shown to have each functional domain of its EII as a separate protein: two soluble proteins (IIA^{Cel} and IIB^{Cel}) that each contain a site of covalent phosphorylation, and one membrane-bound protein (IIC^{Cel}) (321, 322, 383). For PTS-mediated fructose transport in *E. coli* and *S. typhimurium*, a protein called FPr (fructose-specific HPr) was identified (157, 426), which has subsequently been shown to combine the functions of the IIA^{Fru} domain and of an HPr-like protein (133). In this case, then, it is FPr rather than HPr that is phosphorylated by EI (on the HPr-like domain) as an intermediate in fructose translocation. In *Rhodobacter capsulatus*, not only does the fructose PTS combine the domains IIA^{Fru} and HPr but also an EI domain is part of the same protein (542), which may be because only fructose appears to be a PTS carbohydrate in

TABLE 2. Enzymes II of PTSs

PTS	Abbreviation	Organism	Gram stain	Substrates for growth and/or transport[a]	Gene(s)[b]	Domain(s)[b]	No. of residues[b]	Reference(s)
Glucose class								
Glucose	Glc	*E. coli*	−	Glc, GlcN, Sor, αMG, 5TG, Atl, Rtl, Man, 2DG	*ptsG*, *crr*	IICB, IIA	477, 169	105, 416
Glucose	Glc	*S. typhimurium*	−	Glc, αMG, 5TG, Man, 2DG	*ptsG*, *crr*	?, IIA	?, 169	307
Glucose	Glc	*B. subtilis*	+	Glc, αMG	*ptsG*	IICBA	699	140, 142, 549
"Maltose"	Mal	*E. coli*	−	Mal, Glc	*malX*, *crr*	IICB; IIA	530, 169	372
Trehalose	Tre	*E. coli*	−	Tre	*treB*, *crr*	?, IIA	?, 169	27
N-acetylglucosamine	Nag	*E. coli*	−	Nag, Stz, αNag	*nagE*	IICBA	648	336, 338, 399
N-acetylglucosamine	Nag	*K. pneumoniae*	−	Nag, Stz	*nagE*	IICBA	651	511
Sucrose	Scr	*Enterobacteriaceae*	−	Scr, Glc	*scrA*, *crr*	IIBC, IIA	455, 169	89
Sucrose	Scr	*K. pneumoniae*	−	Scr	*scrA*, *crr*	IIBC, IIA	455, 169	244
Sucrose	Scr	*V. alginolyticus*	−	Scr	*scrA*, ?	IIBC, ?	479, ?	24
Sucrose	Sac	*B. subtilis*	+	Scr	*sacP*, ?	IIBC, ?	460, ?	120
Sucrose	Sac	*B. subtilis*	+	Scr	*sacX*, ?	IIBC, ?	459, ?	551
Sucrose	Scr	*S. mutans*	+	Scr	*scrA*	IIBCA	664	433
β-Glucosides	Bgl	*E. coli*	−	Bgl, Glc	*bglF*	IIBCA	625	33, 442
β-Glucosides	Arb	*E. chrysanthemi*	−	Bgl	*arbF*	IIBCA	631	93
β-Glucosides	Asc	*E. coli*	−	Arb, Sal, Cel	*ascF*, ?	IIBC, ?	486, ?	153
Mannitol class								
Mannitol	Mtl	*E. coli*	−	Mtl, Gut, Atl, 2DA	*mtlA*	IICBA	637	235
Mannitol	Mtl	*S. carnosus*	+	Mtl	*mtlA*, *mtlF*	IICB, IIA	505, 145	117, 118, 370
Mannitol	Mtl	*S. faecalis*	+	Mtl	*mtlA*, *mtlF*	IICB, IIA	?, 145	119
Mannitol	Mtl	*S. mutans*	+	Mtl	?, *mtlF*	?, IIA	?, 145	175
Fructose	Fru	*E. coli*	−	Fru, Xtl, Glc, Sor, Man	*fruF*, *fruA*	FPr[c], IIBC	?, 563	367
Fructose	Fru	*S. typhimurium*	−	Fru, Xtl	*fruF*, *fruA*	FPr[c], ?	376, ?	133
Fructose	Fru	*R. capsulatus*	−	Fru	*fruA*	MTP[c], IIBC	827, 578	541, 542
Fructose	Fru	*X. campestris*	−	Fru	?, *fruA*	?, IIBC	?, 580	68
Lactose class								
Lactose	Lac	*L. lactis*	+	Lac, Gal	*lacF*, *lacE*	IIA, IICB	105, 586	84
Lactose	Lac	*L. casei*	+	Lac, Gal	*lacE*, *lacF*	IICB, IIA	577, 112	2, 3
Lactose	Lac	*S. aureus*	+	Lac, Gal	*lacF*, *lacE*	IIA, IICB	103, 572	34
Lactose	Lac	*S. mutans*	+	Lac	*lacF*, *lacE*	IIA, ?	104, ?	410
Cellobiose	Cel	*E. coli*	−	Cel	*celA*, *celB*, *celC*	IIB, IIC, IIA	106, 417, 116	321, 383
Mannose class								
Mannose	Man	*E. coli*	−	Man, Nag, GlcN, Fru, 2DG, Glc, Tre, αMG	*manX*, *manY*, *manZ*	IIAB, IIC, IID	323, 266, 283	107
L-Sorbose	Sor	*K. pneumoniae*	−	Sor, Fru, Glc	*sorF*, *sorB*, *sorA*, *sorM*	IIA, IIB, IIC, IID	135, 164, 266, 274	—[d]
Fructose	Lev	*B. subtilis*	+	Fru	*levD*, *levE*, *levF*, *levG*	IIA, IIB, IIC, IID	146, 163, 269, 275	278
PTS of unknown classification								
Glucitol	Gut	*E. coli*	−	Gut, 2DA, Mtl, Atl	*gutA*, *gutB*	II(CB), IIA	506, 123	544

[a] Abbreviations: Glc, glucose; GlcN, glucosamine; Sor, sorbose; αMG, methyl α-glucoside; 5TG, 5-thioglucose; Atl, arabinitol; Rtl, ribitol; Man, mannose; 2DG, 2-deoxyglucose; Mal, maltose; Tre, trehalose; Nag, *N*-acetylglucosamine; Stz, streptozotocin; αNag, methyl-2-acetamido-2-deoxy-α-D-glucoside; Scr, sucrose; Bgl, β-glucoside; Arb, arbutin; Sal, salicin; Cel, cellobiose; Mtl, mannitol; Gut, glucitol; 2DA, 2-deoxyarabinohexitol; Fru, fructose; Xtl, xylitol; Lac, lactose; Gal, galactose.

[b] ?, not known.

[c] FPr, composed of an EII^{Fru} domain (amino terminal) and an HPr-like domain (carboxy terminal); MTP, multiphosphoryl transfer protein, composed of an EII^{Fru} domain, an HPr-like domain, and an EI-like domain.

[d] Submitted to the EMBL data base by U. F. Wehmeier as accession no. X66059.

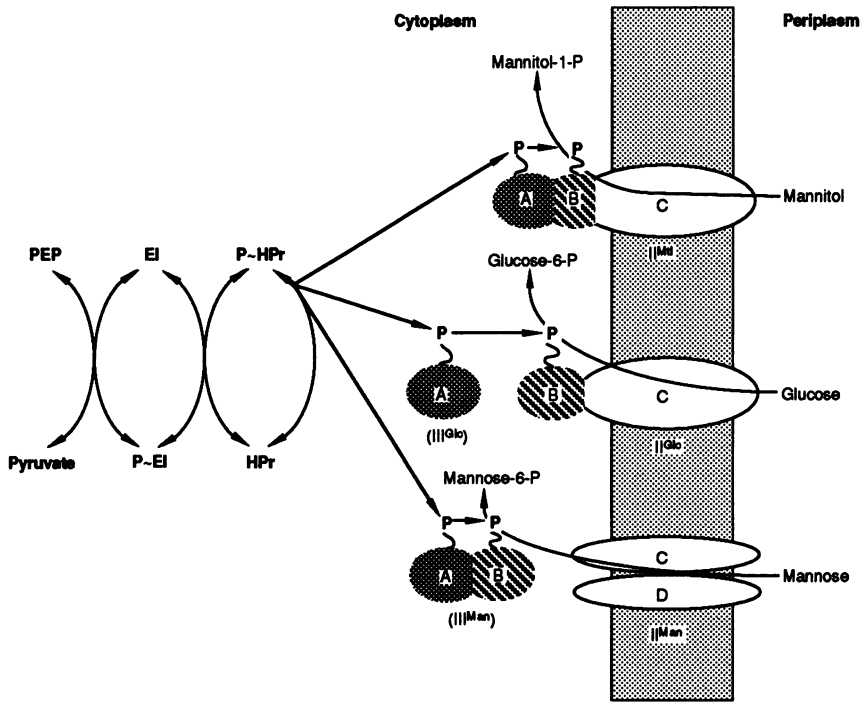

FIG. 1. Organization of PTSs. EI and HPr are the general proteins for all PTSs. Of the many EIIs, only three are shown, those specific for mannitol (Mtl), glucose (Glc), and mannose (Man). Each contains two hydrophilic domains, IIA (formerly EIII or III) containing the first phosphorylation site (P-His), and IIB containing the second phosphorylation site (either a P-Cys or a P-His residue). The membrane-bound, hydrophobic domain IIC may be split into two domains (IIC and IID). II^{Mtl}, II^{Glc}, and II^{Man} are specific for mannitol, glucose, and mannose, respectively. P ~ indicates the phosphorylated forms of the various proteins.

this organism. The domain/polypeptide organizations of over 20 carbohydrate-specific PTSs have been determined, and most fall into one of the categories described above. This topic, as well as a classification based on sequence similarities, is discussed in more detail in the section on structures of the PTS proteins, below.

Purification and Characterization of PTS Proteins

EI. EI has been purified to apparent homogeneity, or partially purified, from a variety of organisms (Table 1). The monomer molecular weight ranges from about 64,000 in *E. coli* and *S. typhimurium* to about 85,000 in *Staphylococcus aureus*. Much evidence suggests that autophosphorylation of EI (reaction 1) requires a dimeric form of the protein (230, 294, 523) and requires divalent cations such as Mg^{2+} or Mn^{2+} (523). The stoichiometry of phospho group incorporation into EI from either *E. coli* or *S. typhimurium* is one per monomer (514, 523), and this reaction requires at least one free sulfhydryl group, which appears to be essential for dimerization (155, 156, 178). Phosphorylation occurs at the N-3 position of a histidyl residue of EI (His-189 in *E. coli*) during autophosphorylation with PEP (4, 523). EI of *S. typhimurium* appears to consist of two domains that can be separated by proteolysis and that are differentially thermostable (256). An amino-terminal, 30-kDa domain containing His-189 participates in reversible phosphorylation of HPr, but the second, carboxy-terminal domain is necessary for autophosphorylation of EI by PEP (256).

The gene encoding EI (*ptsI*) has been cloned and sequenced from *E. coli* (39, 72, 416), *S. typhimurium* (39, 256, 443), and *Staphylococcus carnosus* (218), and partial sequences are known for EI of *Enterococcus faecalis* (5), *S. aureus* (4), and *Bacillus subtilis* (142) (Table 1). In all cases studied there is a high degree of sequence similarity around and including the phosphorylated histidine residue. The deduced amino acid sequences of the EIs from *E. coli* and *S. typhimurium* differ in only 16 residues, consistent with the fact that these proteins are functionally interchangeable in these organisms. However, it has been reported that these EIs and those from certain gram-positive bacteria can only poorly substitute for one another in PEP-dependent phosphorylation of the heterologous HPr (218, 388, 454). In one organism, *R. capsulatus*, EI makes up a domain of a multiphosphoryl transfer protein (MTP) of the PTS, and this domain shares 39% sequence identity throughout most of its length with *E. coli* EI (542).

HPr. HPr (histidine protein) is a small, monomeric protein with a molecular weight of 9,000 to 10,000 except when it makes up a domain of a hybrid, carbohydrate-specific phosphotransfer protein such as in FPr from *S. typhimurium* or the fructose-specific MTP from *R. capsulatus*. Its size and heat stability have aided its purification from a variety of organisms, as listed in Table 1. In addition, it is the first PTS protein whose three-dimensional structure has been studied by X-ray crystallography and solution multidimensional nuclear magnetic resonance (NMR) techniques (see the section on structures of the PTS proteins, below). It is phosphorylated by P-EI at the N-1 position of a histidyl residue (His-15 in *E. coli* HPr [524]), and in all HPrs that have been sequenced (20, 81, 363, 524) or whose sequence has been deduced from that of the *ptsH* gene (39, 72, 91, 142, 416, 490), this His residue and the surrounding sequence are highly conserved.

An examination of HPr sequences shows that HPrs from enteric bacteria lack Trp, Tyr, and Cys residues whereas the HPrs that have been studied so far from gram-positive bacteria contain Tyr. Primary sequence comparisons (385) show that the enteric HPrs are very similar to each other (those from *E. coli* and *S. typhimurium* are identical) but differ considerably from those of gram-positive bacteria except around the His phosphorylation site, which is near the amino terminus of all of these proteins. All HPrs thus far studied from gram-positive bacteria can also be phosphorylated by an ATP-dependent HPr kinase at a Ser residue (Ser-46 in *E. faecalis*) (81, 385), a modification that has been postulated to have a regulatory function (see the section on inducer expulsion in gram-positive organisms, below [79, 82, 376]).

HPr-like domains are also found in the fructose-specific, hybrid phosphotransfer protein FPr from *S. typhimurium* and the fructose-specific MTP from *R. capsulatus* (Table 1). Although these proteins have not been studied extensively in a purified form, they both contain active-site His consensus sequences in their HPr-like domains (135, 542). Moreover, FPr can substitute for HPr in *E. coli* and *S. typhimurium* mutants lacking HPr (46, 134, 275, 426, 513).

Carbohydrate-specific proteins. The first carbohydrate-specific PTS proteins to be studied made up a complex of membrane proteins from *E. coli* that were involved in the phosphorylation of several hexoses (232) and were called II-A and II-B at that time, and two soluble proteins, called factors III, from *S. aureus*, that were necessary, along with membrane proteins, to phosphorylate lactose and mannitol in this organism (128, 162). Subsequently, it was shown that the PTS-mediated phosphorylation of a number of carbohydrates in different organisms required both soluble and membrane-bound substrate-specific proteins (called factors III and enzymes II, respectively). However, once the amino acid sequences of these carbohydrate-specific proteins became known, it was clear that in some cases, there was no separate soluble factor III, but, rather, it comprised a cytoplasmic structural domain of EII that had a factor III-like function (428). A uniform nomenclature for the carbohydrate-specific PTS proteins has been suggested (421) in which factor III or the domain corresponding to it is called IIA and other domains or proteins that make up the carbohydrate-specific complex are called IIB (a second hydrophilic domain), IIC (the membrane-embedded domain), and IID (a second membrane-bound protein found in a few PT systems, e.g., for mannose as described later). This nomenclature will be adopted for the purposes of this review.

The first carbohydrate-specific PTS protein to be purified to apparent homogeneity was the soluble Factor III^{Lac} (IIA^{Lac} in the new nomenclature) from *S. aureus* (162, 447). Its monomer molecular weight was reported to be about 12,000, and its active form was reported to be a trimer of identical subunits. This trimeric structure, however, is possibly an artifact of high protein concentrations and absence of phospholipid in the preparation (77, 458), although recently, IIA^{Lac} from *Lactococcus lactis* has also been proposed to be a trimer (84). Subsequently, the complete amino acid sequence of IIA^{Lac} was determined (473), and the gene encoding IIA^{Lac} (*lacF*) from *S. aureus* was cloned and sequenced (34). IIA^{Lac} is a polypeptide of 103 amino acids (M_r 11,372), consistent with the earlier biochemical studies.

S. aureus IIA^{Lac} was shown to be phosphorylated at the N-3 position of a His residue (apparently His-82) by P-HPr, and to be essential for the PEP-dependent phosphorylation of β-galactosides in this organism (77, 162, 204, 453). However, more recent evidence from site-specific mutagenesis suggests that it is His-78 that is phosphorylated in IIA^{Lac} of *S. aureus* (116), as is apparently also true for IIA^{Lac} from *Lactobacillus casei* (2). Phosphorylation of IIA^{Lac} appears to drastically affect its secondary and tertiary structures, decreasing the α-helical content and apparently exposing more hydrophobic side chains near the surface of the molecule (77). A large conformational change in IIA^{Lac} upon phosphorylation is also supported by the observation that anti-IIA^{Lac} antibodies do not precipitate P-IIA^{Lac} (77).

Undoubtedly the best-characterized IIA protein, however, is that specific for glucose in *E. coli* and *S. typhimurium* (previously called III^{Glc}) that works in conjunction with a membrane-bound IICB^{Glc} protein. This IIA^{Glc}/IICB^{Glc} complex is different from the "II-A/II-B" complex (now called IIAB^{Man}/IIC^{Man}/IID^{Man}) referred to above, which can phosphorylate mannose but also accepts glucose as a substrate. IIA^{Glc} has been purified to homogeneity from both *E. coli* and *S. typhimurium* (286, 368, 445), and its gene (*crr*) has been cloned and sequenced from both organisms (72, 307, 416). The deduced monomer molecular weights (18,230 and 18,226, respectively) agree well with their mobilities on denaturing gels which correspond to M_rs of about 20,000. The deduced amino acid sequences of these proteins differ in only three positions out of a total of 169. Like HPr, IIA^{Glc} is extremely heat stable, and like IIA^{Lac}, it has a strong tendency to form oligomers (446). Like enteric HPrs, it lacks Trp, Tyr, and Cys residues. IIA^{Glc} has also been found in *Vibrio* strains and cross reacts with antiserum against *S. typhimurium* IIA^{Glc} (227, 285, 352).

Enteric IIA^{Glc} is phosphorylated by P-HPr at the N-3 position of His-90 (reaction 3) as an intermediate in PEP-dependent phosphotransfer to glucose (88, 365). It is also required for the uptake and phosphorylation of sucrose in *E. coli* and *S. typhimurium* harboring a naturally occurring plasmid that encodes a IIBC^{Scr} protein (245, 436), and for sugar uptake by the trehalose (27) and the recently discovered, cryptic maltose (372) PTSs in *E. coli*. A number of other soluble IIA proteins have been at least partially characterized (19, 44, 119, 128, 131, 143, 175, 269, 388, 432, 494, 495, 516, 544). Their monomer molecular weights are all in the range of 10,000 to 20,000 with the exception of the hybrid proteins containing a IIA^{Fru} domain (FPr and the MTP from *R. capsulatus*) (133, 542) and the IIAB^{Man} protein involved in mannose transport in *E. coli* (107, 516). In both FPr and the MTP of *R. capsulatus*, the IIA^{Fru} domains are amino-terminal, possibly because, in general, it is the amino-terminal region of each IIA protein that interacts with the IICB complex. In IIAB^{Man} a hydrophilic IIB domain that is usually part of a membrane-bound IICB complex is instead covalently linked to the IIA domain. This protein has been purified (107), has a monomer molecular weight of 35,000, and has been shown to be phosphorylated on two different histidyl residues, one in each domain (106). His-10 in the amino-terminal domain (IIA or P13) is phosphorylated at the N-3 position by P-HPr, and this phospho group is then transferred to the N-1 position of His-175 in the carboxy-terminal domain (IIB or P20). In addition, IIAB^{Man} appears to be active as a dimer (106).

For a number of PTS permease complexes the IIA portion has been shown to be covalently linked to the other domains, IIB and IIC. This was first inferred to be the case for II^{Mtl}, the EII specific for mannitol in *E. coli*, because the protein purified from the membrane in detergent solution did not require any soluble proteins other than EI and HPr to phosphorylate mannitol in vitro (187). Other examples of this

situation include II^{Nag} (specific for *N*-acetylglucosamine) in enteric bacteria (338, 399, 511), II^{Bgl} (specific for β-glucosides) in *E. coli* (32, 442), II^{Scr} in *Streptococcus mutans* (433), and II^{Glc} of *B. subtilis* (140, 476, 549). With the exception of IIA^{Mtl}, these IIA domains are highly similar in primary structure to the separate IIA^{Glc} proteins of *E. coli* and *S. typhimurium*, especially in the region surrounding the active-site His residue (see the section on structures of the PTS proteins, below). In contrast, the IIA^{Mtl} domain of *E. coli* is similar in structure to the separate IIA^{Mtl} proteins of *S. carnosus*, *S. aureus*, *S. mutans*, and *E. faecalis* (117, 119, 175, 370), as well as to the IIA^{Fru} domains of the hybrid proteins FPr and the MTP of *R. capsulatus* (133, 542). In IIA^{Mtl}, His-554 of the multidomain IICBA protein is phosphorylated by P-HPr and highly similar regions are found in its relatives as well (see the section on structures of the PTS proteins, below).

The first carbohydrate-specific PTS protein containing the integral membrane domain (IIC) to be purified to apparent homogeneity in a functional form was II^{Mtl} of *E. coli* (187). This purification made use of the fact that the nonionic detergent Lubrol-PX interacts strongly with II^{Mtl} and could be used to specifically elute this protein from a hexyl-agarose column. This 65,000-Da protein catalyzed the PEP-dependent phosphorylation of mannitol in vitro in the presence of purified EI and HPr, without an apparent requirement for other soluble factors. Subsequent characterization of the purified protein showed that it was extremely sensitive to proteolysis by trypsin, which cleaved the protein nearly in half (186). Studies of the membrane-bound protein showed that it also could be cleaved by trypsin but only in everted membrane vesicles (185, 467). A 29-kDa fragment was released from these vesicles by trypsin treatment and was shown to be the carboxy-terminal half of the protein, whereas a 34-kDa amino-terminal fragment remained associated with the membrane (467). The gene encoding *E. coli* II^{Mtl} (*mtlA*) was cloned and sequenced, and a hydropathy analysis of the primary sequence showed the amino-terminal half to be very hydrophobic and the carboxy-terminal half to be hydrophilic (235), in accord with the direct topographical studies.

Phosphorylation of purified II^{Mtl} by P-HPr has been directly demonstrated (404, 516), with a stoichiometry of two phospho groups incorporated per polypeptide chain (329). As will be discussed below, considerable evidence suggests that His-554 in the IIA domain and, surprisingly, a cysteinyl residue (Cys-384) in the IIB domain are the sites of phosphorylation, in sequence, during phosphotransfer to mannitol. His-554 is probably phosphorylated at the N-3 position of its imidazole ring (326, 328).

The integral membrane EIIs specific for glucose from *E. coli* (29, 288) and *S. typhimurium* (29, 103) have also been purified to apparent homogeneity from detergent-extracted membranes, and their properties are very similar. Both have apparent subunit molecular weights of 45,000 by denaturing gel electrophoresis. Thus, the sum of the molecular weights of these proteins (which we now know consist of IIB and IIC domains) and that of IIA^{Glc} is about 65,000, nearly identical to that determined for $IICBA^{Mtl}$. Purified $IICB^{Glc}$ catalyzes the PEP-dependent phosphorylation of glucose and methyl α-D-glucopyranoside (αMG) in the presence of EI, HPr, and soluble IIA^{Glc}. $IICB^{Glc}$ is phosphorylated by P-IIA^{Glc} in a reaction necessary for glucose phosphorylation (15, 99). The site of phosphorylation of $IICB^{Glc}$ is also a cysteine residue in the IIB domain (Cys-421) (102, 287a). The sequence surrounding this residue shows weak similarity to that surrounding Cys-384 of EII^{Mtl} (328).

The first integral membrane EII complex to be characterized was the II^{Man} complex of *E. coli* (then called II-A and II-B [232]), which has a rather broad substrate specificity for transport and phosphorylation (Table 2). Much later (105), this complex was highly purified in a functional form from an overproducing strain. Four bands were observed on a polyacrylamide gel in this preparation, with mobilities corresponding to molecular masses of 60, 35, 27, and 25 kDa. The 35-kDa protein was shown to be the soluble $IIAB^{Man}$ monomer (also see above), the 60-kDa band was the dimeric form of this protein, and the 27- and 25-kDa proteins were shown to be integral membrane proteins. In this work, it was suggested that the 25-kDa protein was related to the 27-kDa polypeptide, possibly through proteolysis (104). Subsequent sequencing of the entire transcriptional unit involved in mannose transport, however, showed that it consists of three contiguous genes (then called *ptsL*, *ptsP*, and *ptsM* [107]; now called *manX*, *manY*, and *manZ*, respectively (421, 529]), which encode $IIAB^{Man}$, and two membrane proteins that were called II-P^{Man} and II-M^{Man}, respectively. The deduced molecular masses of II-P^{Man} (now called IIC^{Man}) and II-M^{Man} (now called IID^{Man}) were 28 and 31 kDa, respectively. The 25- and 27-kDa proteins purified in the earlier work were shown to be the products of the *ptsP* (*manY*) and *ptsM* (*manZ*) genes and undoubtedly run faster on denaturing gels than expected on the basis of their true molecular weights because they are both enriched in hydrophobic residues (107).

All three proteins, $IIAB^{Man}$, IIC^{Man}, and IID^{Man}, are required for PEP-dependent carbohydrate phosphorylation by the II^{Man} complex (107, 431, 529). As mentioned above, the $IIAB^{Man}$ protein is phosphorylated at histidyl residues (His-10 and His-175) in each of its domains, which have been isolated after proteolytic cleavage of intact $IIAB^{Man}$ (106). There is no evidence for P~Cys in II^{Man}, although the IIC^{Man} protein contains a cysteinyl residue around which there is some similarity in sequence to the Cys-384 region in II^{Mtl} (326).

Compared with the enteric II^{Mtl}, II^{Glc}, and II^{Man} proteins/complexes, much less is known about the biochemistry of the integral membrane complexes of other PTSs, partly because none of these has been studied in a purified, functional form. Most of our information on these PTSs (Table 2) comes from deduced amino acid sequences and similarities to the better-studied systems, as discussed in the following section.

STRUCTURES OF PTS PROTEINS

Many nucleotide sequences for genes encoding different PTS proteins and their deduced amino acid sequences have become available in recent years (Tables 1 and 2). Fewer PTS proteins have been purified and analyzed by physicochemical methods; however, the three-dimensional structures of a few of the soluble PTS proteins are known. To date, models of the structures of the membrane-bound EIIs have relied on secondary-structure predictions or gene fusion techniques (274). All such models, of course, must be validated experimentally by different biochemical and genetic tests. In this section, we review recent evidence about the structures of PTS proteins as they relate to each other and to their functions.

General PTS Proteins

EI. Complete and partial amino acid sequences, as deduced from DNA sequencing, have been determined for a variety of EIs and EI domains as listed in Table 1. In addition, local sequence similarity in EI was found with two other enzymes, pyruvate phosphate dikinase (346, 542) and PEP synthase (312), which can autophosphorylate at an active-site histidine with PEP or ATP as the phospho donor. The four local similarities include the region of the phosphorylated histidine in EI (4) and thus probably define the catalytic site for PEP binding and phosphotransfer.

According to present models (155, 156, 310), EI from enteric bacteria associates at room temperature to an active dimer of identical subunits, which in the presence of Mg^{2+} and PEP is phosphorylated on each subunit. These dissociate, especially at lower temperatures, to the monomers which phosphorylate HPr. The subsequent reassociation of subunits is apparently the rate-limiting step of the whole cycle. Microcalorimetry (256) and fluorescence spectroscopy (310) indicate the existence of two autonomous domains, possibly connected through a linker, whose cooperative interactions are strongly pH and temperature dependent. The domains can easily be separated in an active form by protease treatment. The amino-terminal domain alone, containing the active-site His, cannot be phosphorylated by PEP or by P-EI but can be phosphorylated by P-HPr in a fully reversible reaction. This domain, which apparently interacts with HPr, is less conserved among the different EIs, pyruvate phosphate dikinase, and PEP synthase than is the carboxy-terminal domain. The latter thus seems to be involved primarily in the interaction with PEP and in dimer formation and consequently may play a key role in regulating EI activity (256).

HPr. The primary amino acid sequences of HPr from a series of bacteria have been determined (Table 1). They show a considerable degree of conservation, especially around the histine residue that is phosphorylated. Moreover, HPrs from *E. coli* and *S. typhimurium* are identical (39, 72, 75, 363, 416, 443), and only one conservative exchange (I63L) was found in HPr from *Klebsiella pneumoniae* (490). As mentioned above, however, HPrs from gram-positive bacteria, although highly similar to each other, do differ considerably in amino acid sequence from the gram-negative proteins outside of the region containing the phosphorylated His residue (385).

The three-dimensional structure of HPr has been investigated by several independent methods: two-dimensional NMR (154, 207, 212–214, 533, 534) and three-dimensional NMR (500) of HPr in solution and X-ray crystallography (94, 172). The structure of the *E. coli* protein determined by the NMR studies consists of four β-strands that form a single, pleated antiparallel β-sheet on one face of the protein and three α-helices that lie in a plane parallel to the β-sheet. Further secondary structures are four reverse turns and regions of extended backbone structure. According to the X-ray structure of the *E. coli* protein, however, the four β-strands exist in two pairs removed from each other and the α-helices lie in different planes and orientations, whereas the region containing the phosphorylated His residue was the same in both studies. The recently determined crystal structure of *B. subtilis* HPr to a resolution of 2.0 Å (0.2 nm) (172) agrees with the overall folding pattern of the *E. coli* protein determined by the NMR methods. In this structure, *B. subtilis* HPr is folded as an open-faced β-sandwich formed by four antiparallel β-strands and three apposed α-helices. Furthermore, 34 of the 85 residues of *E. coli* HPr were changed by mutagenesis and alterations in binding to monoclonal antibodies were determined (449). Altered binding defines the residues that form exposed epitopes of HPr and thus helps to discriminate between the two structures. Only the NMR-derived model for *E. coli* HPr (and the similar structure determined for the *B. subtilis* protein) is consistent with the results from this epitope mapping. Thus, the X-ray structure of HPr from *E. coli* may represent a partially unfolded state that was favored by the crystallization conditions (94, 449).

All of the structural studies have confirmed previous reports (205, 206, 385) that had established an essential role for His-15 (the phosphorylated residue) and for Arg-17. His-15 is surface localized in the *B. subtilis* X-ray structure, capping the amino terminus of the first α-helix. The nearby Arg-17 is separated from His-15 in this structure by a sulfate ion, which forms a salt bridge with the guanidinium group of Arg-17. This prompted speculation that this sulfate ion may mimic the phospho group in phospho-HPr and that Arg-17 could thus form a salt bridge with the phospho group in P-HPr (172). Although an essential role of the carboxy-terminal Glu-85 in *E. coli* HPr has been postulated (10), the structure of the *B. subtilis* protein shows no obvious explanation for this observation (172). Finally, Ser-46 in *B. subtilis* HPr is also surface localized, capping the amino terminus of the second α-helix of the structure (172). It is this residue in gram-positive HPrs that is phosphorylated by an ATP-dependent kinase, and its putative role and the structural changes which accompany seryl phosphorylation (533) are discussed in the section on interactions between PT and non-PT systems, below.

Classification and Functional Domains of PTS EIIs

As discussed above, EIIs may consist of a single membrane-bound protein or of two to four proteins at least one of which is membrane-bound. As summarized schematically in Fig. 2, each EII is composed of three domains (IIA, IIB, and IIC). With the possible exception of EIIs in the mannose class (see below and Table 2), the variations in the numbers of proteins that make up a given EII complex are solely due to fusion or splitting of these domains during evolution but do not reflect mechanistic differences.

On the basis of sequence alignments, EIIs may be grouped into at least four classes (Table 2) (248, 374, 421, 428). The amino acid sequences of the EIIs belonging to one class share more than 25% identical amino acid residues over the entire molecule, while the similarities for members of different classes are restricted to more local motifs. Furthermore, whereas complementation between equivalent domains of the members of a class is high, IIA^{Glc} or IIA^{Nag} from the glucose class are unable to complement the lack of IIA^{Mtl} in the mannitol class, for example. Lack of interclass complementation most probably reflects the existence of specific interactions between the different domains.

Most EIIs share several conserved features (Fig. 2) (242): (i) three autonomous structural domains IIA, IIB, and IIC, which may be free or fused, comprising a total of about 630 residues (if fused, they are coupled by flexible linkers and may be arranged in different orders); (ii) a hydrophilic domain IIA (ca. 100 residues) with a conserved His residue which can be phosphorylated by P-HPr; (iii) a hydrophilic domain IIB (ca. 100 residues), which may be on either side of IIC and is phosphorylated by IIA at a conserved cysteine; and (iv) a IIC domain of about 350 residues, which includes

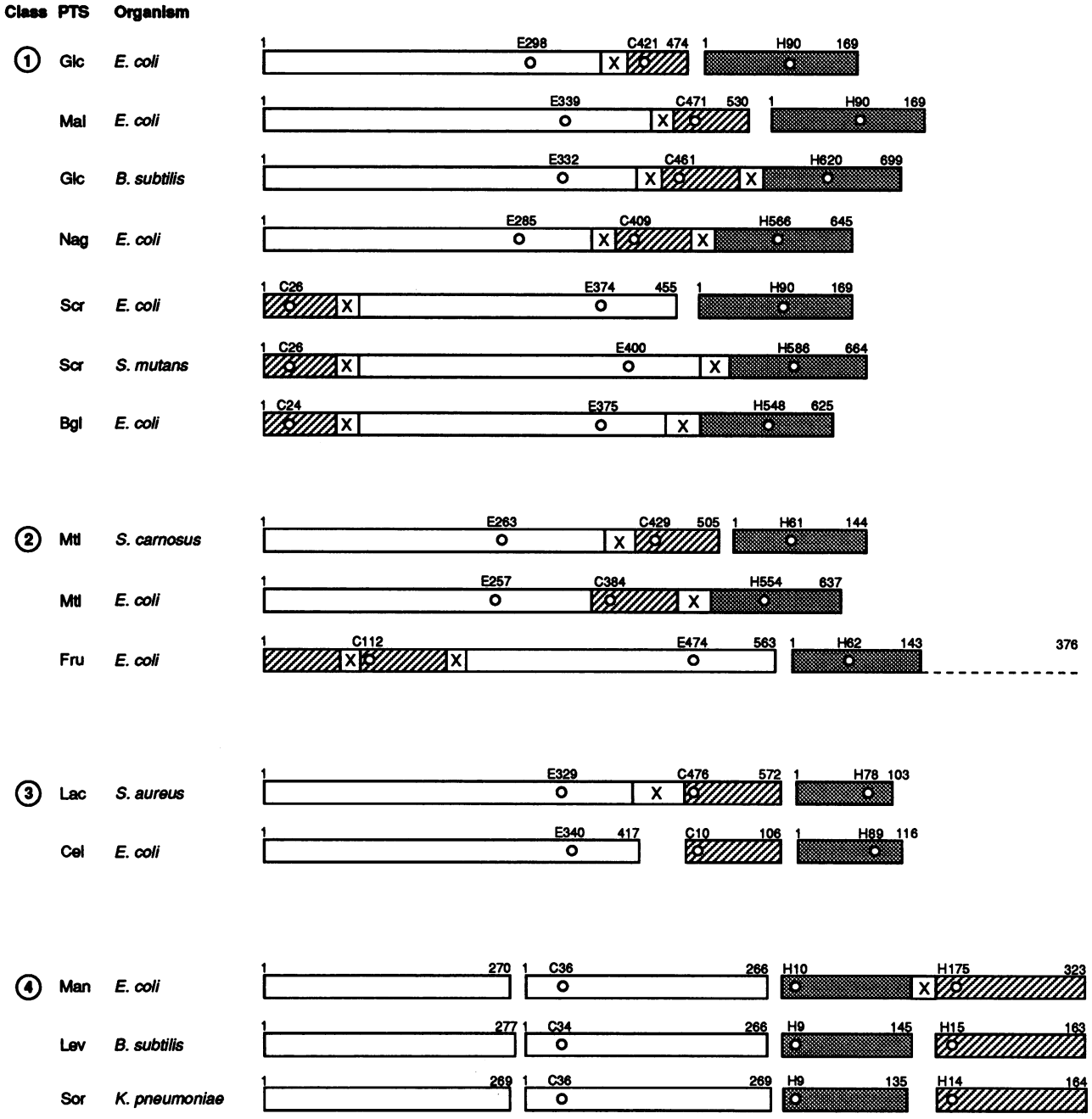

FIG. 2. Arrangement of the domains IIA, IIB, IIC, and IID within the four classes of EIIs. The various EIIs can be divided into four classes, each class consisting of genetically related proteins with more than 20% identical residues over the length of the molecule(s). Symbols: stippled boxes, IIA; hatched boxes, IIB; white boxes, IIC and IID; ×, presence of a linker; ○, essential residues, i.e., the phosphorylated histidine (H) of IIA (and of the IIB domains of class 4), the phosphorylated cysteine (C) of IIB, and the glutamate (E) of IIC. Numbers indicate the first (number 1) and the last residues of the different peptides as well as the position of the essential residues within each polypeptide. Other abbreviations are as defined in Table 2. For FPr (376 residues), only the IIA domain is indicated.

six to eight potential transmembrane helices and at least one large hydrophilic loop. This loop always includes a histidine and a GXXE motif (see below).

In most cases, these domains are connected by linkers (for a review, see references 335 and 539). Several types of linkers have been described: (i) the PA linker, which consisting of Pro-Ala repeats interspersed with charged residues; (ii) the Q-linker, which contains hydrophilic residues (Gln, Asn, and His) and Ser, Thr, and Ala; and (iii) a general linker, which contains a number of charged residues.

Glucose class. II^{Glc} of *E. coli* consists of two proteins, the soluble IIA^{Glc} (encoded by *crr*) and the $IICB^{Glc}$ protein (encoded by *ptsG*). According to a hydropathy plot analysis, IIC^{Glc} corresponds to the membrane-bound domain which constitutes the amino terminal part of the molecule, while the carboxy terminal IIB^{Glc} domain is hydrophilic. These are

joined by a linker (248). Since IIB^{Glc} is phosphorylated at a Cys residue by the soluble and intracellular IIA^{Glc} (102, 287a), it must be accessible to this phospho-carrier protein at the inner site of the cytoplasmic membrane. Protein sequence alignments show that a $IICB^{Mal}$ from *E. coli*, which relies on IIA^{Glc} for its activity, is closely related to $IICB^{Glc}$ of *E. coli* (34.9% identical residues [372]), as are the II^{Glc} from *B. subtilis* (44% identical residues [549]) and II^{Nag} from enteric bacteria (44% identical residues [338, 399, 511]). In the last two, IICB is fused to IIA through a linker (476, 511).

On the basis of primary amino acid sequence similarities (33, 89, 120) and of complementation tests with heterologous domains (388, 440, 441, 507, 509) and intergenic hybrids (179), several EIIs specific for sucrose and β-glucosides also belong to the glucose class. However, those sequences are more closely related to each other than to other members of the class. Within the II^{Scr}-II^{Bgl} subclass, IIB is located at the amino-terminal end, fused to IIC through a linker. If, as discussed shortly, domain IIC is normally cylindrical, the IIBC and IICB combinations could be structurally equivalent. The $IIBC^{Scr}$s from enteric bacteria (245) and *B. subtilis* (120, 388, 476) both require IIA^{Glc} for activity. This example of "cross-talk" between different carbohydrate-specific PTSs further corroborates the close linkage between all members of the glucose class. In II^{Scr} from *S. mutans* (433) and several EIIs specific for β-glucosides (Table 2), the IIB, IIC, and IIA domains are fused into a single polypeptide, and the IIC and IIA domains are joined by a linker. Thus, the IICBA and IIBCA proteins correspond to a complex in which the three domains have been fused to a single protein and are encoded in a single gene. Furthermore, nucleotide and amino acid sequence differences between closely related and fused EIIs, e.g., II^{Nag} from *E. coli* and *K. pneumoniae*, are more frequent within the linkers (511). Taken together, these observations strongly support a modular evolution of the glucose class of EIIs from autonomous protein domains. The interdomain DNA then corresponds to recombination sites allowing variable arrangements of gene fragments, and the corresponding protein sequences correspond to protein linkers.

Mannitol class. II^{Mtl} from *E. coli*, perhaps the best-understood EII, shows only local similarity to members of the glucose class (248). It is a large single protein of the domain structure IICBA. The membrane-embedded IIC domain carries out substrate binding and translocation, and the cytoplasmic IIA and IIB domains contain the His and Cys phosphorylation sites, respectively (147, 262, 328, 329). Various combinations of these domains have also been cloned and expressed in functional forms in *E. coli*, confirming their structural and functional independence (147, 504, 505, 527). The results establish IIC (residues 1 to 334) as an autonomous domain sufficient to bind the substrate and to catalyze its translocation. The presence of IIB (residues 335 to 457) with an intact Cys-384, however, is needed for vectorial phosphorylation in the presence of an intact IIA domain (residues 458 to 637) containing His-554 (506, 527). In contrast to the situation in *E. coli*, mannitol PTSs from several gram-positive bacteria have separate IICB and IIA proteins (Fig. 2) (117–119, 175). Nonetheless, these proteins show significant sequence similarity to the corresponding parts of the *E. coli* $IICBA^{Mtl}$ protein, and IIA^{Mtl} of *S. carnosus* can replace a nonfunctional IIA^{Mtl} domain of the *E. coli* protein (505).

Several II^{Fru} complexes, which on the basis of sequence similarities clearly belong to the mannitol class (Table 2), have been sequenced and analyzed recently (68, 69, 133, 367, 541). However, their domain structures are unusual. In contrast to previously published results (475), it was found that in enteric bacteria a soluble protein (376 residues) called FPr contains three domains (133): (i) an amino-terminal IIA^{Fru}, which resembles IIA^{Mtl} of other mannitol PTSs (approximately 38% identical residues); (ii) a central part of unknown function with a weak similarity to the receiver part of the two-component systems (542); and (iii) a carboxy-terminal part, which resembles *E. coli* HPr (35% identical residues). In transport complementation tests, FPr can substitute efficiently for HPr, hence its previous name "pseudo-HPr" (51, 157, 426). As expected, the corresponding membrane-bound II^{Fru} protein contains domains IIB and IIC. With 553 residues, however, *E. coli* II^{Fru} is much larger than expected for a protein containing only IIB and IIC domains. This is due to an internal duplication (residues 1 to 100 and 116 to 215) fused through a linker (541). Interestingly, II^{Fru}s from *R. capsulatus* (541) and from *Xanthomonas campestris* (68) are closely related in sequence (ca. 30% identical residues) and structure to *E. coli* II^{Fru}. In each, only the second duplication contains a conserved cysteine residue, which resembles the Cys-384 region of *E. coli* IIB^{Mtl} as a potential phosphorylation site.

In *R. capsulatus*, the MTP is involved in fructose PTS activity. It contains, in a fused form (827 residues), an amino-terminal IIA^{Fru} domain (residues 1 to 143), an HPr-like domain (residues 157 to 245), and an EI-like domain (residues 273 to 827), all domains being coupled through linkers (542). This arrangement not only is another example of the modular evolution of the PTS from autonomous domains but also supports the notion that all PTS domains can act as a complex enzyme unit at the inner surface of the cytoplasmic membrane.

The entire fructose PTS (ca. 630 residues after deduction of the IIB duplication and the non-IIA parts of FPr or MTP) fits the conventional scheme. Whereas the IIA^{Fru} and IIB^{Fru} domains resemble the corresponding II^{Mtl} domains, the IIC^{Fru} domain seems more closely related to IIC^{Scr} and IIC^{Bgl} (541). Perhaps this indicates an exchange of modules during evolution between the different EII classes.

Lactose class. *S. aureus* (34), *L. lactis* (84), and *L. casei* (3) contain a lactose PTS (47 to 72% identical residues), which consists of a soluble IIA domain and a membrane-bound IICB complex. IIB^{Lac} contains a highly conserved and essential cysteine residue. Furthermore, sequence alignments showed that these lactose PTSs closely resemble (25 to 35% identical residues) a cellobiose PTS encoded by a cryptic *cel* operon in *E. coli* (321). Three Cel proteins, IIB^{Cel} (*celA*), IIC^{Cel} (*celB*), and IIA^{Cel} (*celC*), constitute the equivalent of $IIBC^{Lac}$ and IIA^{Lac} of the lactose PTS (383).

Mannose class. Three highly similar PTSs (45 to 65% identical residues), for mannose (107) and L-sorbose (535) (EMBL data base accession number X66059; submitted by U. F. Wehmeier) in enteric bacteria and for fructose in *B. subtilis* (278), are an exception to the general scheme (Fig. 2). Each can phosphorylate fructose in addition to its major substrate. II^{Man} from *E. coli* and II^{Fru} from *B. subtilis* generate fructose 6-phosphate, and II^{Sor} generates fructose 1-phosphate. The mannose PTS consists of three polypeptides (107, 529). A soluble protein, ManX ($IIAB^{Man}$; 323 residues) contains two domains, P13 and P20, fused by a linker (100, 106). These domains correspond functionally to IIA and IIB domains, respectively. According to sequence alignments (278) and to complementation tests for the sorbose PTS (535), $IIAB^{Man}$ corresponds to two proteins in the two other systems. These are LevD (IIA^{Fru}; 146 residues)

and LevE (IIB^{Fru}; 163 residues) for the fructose PTS of *B. subtilis*, and SorF (IIA^{Sor}; 135 residues) and SorB (IIB^{Sor}; 164 residues) for the L-sorbose PTS of enteric bacteria. Deletions of the genes *sorF* and *sorB* can be complemented in vivo and in vitro by $IIAB^{Man}$. In both systems, the reading frames of the two genes overlap, usually an indication for a tight transcriptional coupling. As expected, the characteristic linker which fuses P13 (IIA^{Man}) and P20 (IIB^{Man}) in $IIAB^{Man}$ is not present in the other two systems.

Two proteins constitute the membrane-bound part of this class of EIIs. One, EIIC (ManY [IIC^{Man}], LevF [IIC^{Fru}], and SorA [IIC^{Sor}]), is strongly hydrophobic and contains at least four transmembrane helices distributed throughout its entire length. The second, EIID (ManZ [IID^{Man}], LevG [IID^{Fru}], and SorM [IID^{Sor}]), is a hydrophilic or moderately hydrophobic protein whose carboxy-terminal part contains two putative transmembrane helices. Neither IIC^{Man} nor IID^{Man} seems to be phosphorylated, but all three proteins of the mannose PTS are required for the vectorial phosphorylation of mannose (107, 529).

Are all PTSs in this class phosphorylated on two His residues rather than a His and a Cys residue? Both His-10 and His-175, the residues phosphorylated in $IIAB^{Man}$, are conserved in the equivalent domains of the sorbose and the fructose PTSs. These domains seem functionally equivalent since the lack of IIA^{Sor} and IIB^{Sor} can be complemented by $IIAB^{Man}$ (535). It has been pointed out (326) that the region around Cys-16 in IIC^{Man} is rather similar to that surrounding Cys-384 in EII^{Mtl}. This Cys-16 is conserved in IIC^{Sor} but not in IIC^{Fru}. However, another Cys residue (Cys-36 in IIC^{Man}) is conserved in all three domains. The low sensitivity of the mannose PTS to *N*-ethylmaleimide, observed previously (152), could thus be due to the protection of an essential Cys residue in the membrane or to the fact that His-175 is its functional equivalent.

The mannose PTS is required for infection of *E. coli* cells by bacteriophage λ (95, 104, 431, 529) as well as phage N4 (210). The hydrophilic amino-terminal part of IID^{Man} seems essential in phage λ recognition, and it may protrude into the periplasmic space (107). Neither $IIAB^{Man}$ nor phosphorylation is essential for phage λ infection, perhaps indicating the formation of a transmembrane channel or pore by the two membrane-bound proteins. It is not clear at present whether IIC^{Man} alone is sufficient for phage λ infection (529) or whether both IIC^{Man} and IID^{Man} are required (107).

Glucitol PTS. Although glucitol is structurally related to mannitol and although both polyhydric alcohols are taken up by both PTSs (238), the EIIs lack similarity. The glucitol PTS of *E. coli* contains a soluble IIA domain and an unusual membrane-bound protein in which two hydrophobic parts (residues 1 to 156 and 332 to 506) are separated by a very large hydrophilic loop (residues 156 to 332 [544]). Because even local similarity between EII^{Gut} and any other class of EIIs cannot be found, at present it is impossible to propose any domain structure.

Structures of IIA and IIB Domains

The secondary structure of the IIA^{Glc} protein from *E. coli* was first determined by heteronuclear (^{15}N, ^{13}C) three-dimensional NMR spectroscopy (333, 334). These results showed extensive antiparallel β-sheet structures, with the two essential His residues (His-75 and the phosphorylated His-90) in close proximity but with the first 18 amino-terminal residues highly disordered in solution. It was speculated that the amino-terminal part of IIA^{Glc} adopts a more ordered structure on interaction with IIB^{Glc} and/or the cell membrane. Three-dimensional NMR studies gave a similar structure for the IIA^{Glc} domain from *B. subtilis* (162 residues) that had been purified after subcloning the part of the *ptsG* gene coding for this domain (108, 109). These authors assign the flexible structure of the amino-terminal part to the fact that it is part of a linker as noted before (476). Further structural details of IIA^{Glc} from *B. subtilis* and *E. coli* have recently become available from X-ray crystal structures of the two related proteins (41% identical residues [254, 540]), most importantly the spatial arrangement of 28 conserved residues, many associated with the active site. Overall, the molecules have a β-barrel or β-sandwich structure consisting of 12 β-strands, with loops, short β-strands, and distorted α-helices connecting the strands. In both structures, the active-site (phosphorylated) His residue (His-83 in *B. subtilis* IIA^{Glc} and His-90 in *E. coli*) lies in a shallow hydrophobic depression on the surface of the β-structure. A second His residue (His-68 in *B. subtilis* and His-75 in *E. coli*) is located at the active site with its N-3 atom 3.2 to 3.3 Å (0.32 to 0.33 nm) from the N-3 atom of the active-site His in both structures. Both X-ray structures suggest that when His-83/90 is phosphorylated, the phospho group also interacts with His-68/75. A role for His-68/75 in phosphotransfer from the active-site His residue to IIB^{Glc} had been proposed on the basis of site-directed mutagenesis studies (365, 388), and its possible role in the mechanism of phosphotransfer is discussed in the next section. Other conserved residues form the hydrophobic surface patch around the active-site His, which has been proposed to be important for the recognition of HPr and/or the corresponding IIB domain (248, 254, 540).

Finally, the structure of the phosphorylated and unphosphorylated forms of IIA^{Glc} have been compared (110, 332, 472). The data show that only small structural rearrangements limited to the active site occur on phosphorylation, thus confirming the results from an earlier study (88). In contrast to the minor structural changes observed for the IIA^{Glc} domains, large conformational changes have been observed on phosphorylation of IIA^{Lac} from *S. aureus*, as mentioned in the section on carbohydrate-specific proteins, above. The recent crystallization of IIA^{Lac} from *S. aureus* (42) and the IIA domain of $IIAB^{Man}$ of *E. coli* (136) should allow more detailed analyses of the possible differences between phosphorylated and unphosphorylated forms of IIA proteins and domains, as well as of regions in these molecules that interact with P-HPr.

In contrast to the IIA domains and proteins, there is little structural information available on the IIB domains containing the Cys phosphorylation site. Since it should be possible to express and purify these domains from various EIIs, structural details of IIB should be forthcoming in the near future.

Structures of IIC Domains

Secondary-structure models have been proposed for the membrane-bound IIC domains which rely primarily on prediction methods, the location of strictly conserved amino acid residues, and gene fusion techniques. These predict that IIC domains of the glucose class may span the membrane six or eight times and that IIC^{Mtl} of *E. coli* has at least six identifiable transmembrane stretches. The latter structure has been tested with a series of PhoA fusions spread evenly over the entire domain (474). The analysis provides strong evidence for six membrane-spanning structures, most probably α-helices of about 20 residues. There are three short

periplasmic loops (loops 2, 4, and 6) and two large cytoplasmic loops (loops 3 and 5). Loops are numbered from the amino terminus, with the first 24 residues as loop 1. Both of the large loops, and especially loop 5, are very highly conserved in at least one other IIC^{Mtl} domain that has been sequenced (118). As discussed in the section on mechanism and regulation of transport catalyzed by the EIIs (below), loop 5 in II^{Mtl} and an equivalent region in *E. coli* II^{Glc} may be critical for the activities of these proteins. The model for IIC^{Mtl} differs somewhat from one proposed before (189) but is consistent with secondary-structure predictions and biochemical studies on which the former model was based (474).

Of the ca. 120 residues of IIC^{Mtl} located in putative membrane-spanning helices, only 20 are polar and/or capable of forming hydrogen bonds with the substrate (474), a property the model shares with models proposed for other EIIs (244). This limits the number of residues that could contribute to the substrate binding and translocation sites of a channel, as well as the number of possible three-dimensional arrangements of the transmembrane structures. On the basis of simple geometric considerations, a bundle of at least five transmembrane amphipathic α-helices are predicted to be necessary to form a channel large enough to admit a carbohydrate (189). Since, however, only two highly amphipathic transmembrane helices may be present in IIC^{Mtl} (474), an oligomer might be necessary to form the channel. Evidence to be discussed in the section on oligomerization and evidence for intersubunit phosphotransfer (below) strongly argues for oligomerization of EIIs through their IIC domains and suggests that this oligomeric form of the EIIs is the predominant species under physiological conditions.

For $IICB^{Glc}$, a *phoA/lacZ* fusion study indicates eight instead of six transmembrane helices in the IIC^{Glc} domain and places a region corresponding to part of hydrophilic loop 5 in IIC^{Mtl} into the membrane (37a). It remains to be shown whether these EIIs differ in structure in this part of the molecule or whether the models are not yet accurate.

As in many other EIIs (105, 428), the extreme amino-terminal part of IIC^{Mtl} (residues 1 to 23) is able to form an amphipathic helix that is probably involved in targeting and inserting transmembrane helix I into the membrane (36, 349, 547). Since some EIIs have the intracellular IIB domain fused to the amino terminus of a similar amphipathic helix (Fig. 2), it seems reasonable to propose that these potential membrane-targeting helices are cytoplasmic rather than transmembrane. Stable anchoring to the membrane is also dependent on other structural elements of the IIC domain, in particular transmembrane helices 5 and 6 in IIC^{Mtl} (36).

Finally, EIIs of the mannose class (Table 2) also seem to form a stable membrane-associated complex in which the soluble protein(s) associate(s) in a reversible form with the two membrane-bound proteins as has been shown for the mannose-PTS (100, 104, 106, 529). Fusion studies indicate that the carboxy-terminal end of IIC^{Man} (probably four transmembrane helices) is close in space to the amino-terminal end of the second membrane-bound protein IID^{Man} (two helices), giving a six-helix structure (448).

MECHANISMS OF PTS-MEDIATED TRANSPORT AND PHOSPHORYLATION

In the section on overview and PTS components (above), we considered the overall phosphotransfer reactions responsible for PTS-mediated carbohydrate phosphorylation and properties of the proteins responsible. In this section, we review recent work on the detailed mechanisms of the phosphotransfer reactions catalyzed by the PTS, the mechanism by which phosphorylation is (or is not) coupled to transmembrane translocation, and structure-function relationships in the EIIs that may account for these mechanisms.

Phosphotransfer Involving EI and HPr

Reactions 1 through 5 (see above) describe the sequence of phosphotransfer reactions in all PTSs for which this has been studied in detail. As discussed in that section, P~His is the protein-bound phospho intermediate for EI and HPr. Steady-state kinetic (518, 525) and isotope exchange (177) studies are consistent with P-EI being a bona fide intermediate in reactions 1 and 2. For EI from *S. typhimurium*, the K_m for PEP for phosphorylation of HPr is 0.2 to 0.4 mM (424, 525), and that for HPr is about 5 μM (525). Similar K_m values were obtained for EI from *E. coli* (388, 518). As expected, EI catalyzes a phospho-exchange reaction between PEP and pyruvate (177, 424) that has been used as a convenient assay for EI. The phosphoryl transfer potential of P-EI is nearly as high as that of PEP (523), and, in addition to its ability to phosphorylate HPr at a His residue (see below), P-EI can reversibly phosphorylate acetate kinase from either *E. coli* or *S. typhimurium* at a Glu residue (124). The significance of the latter observation is unknown, but it has been postulated to be a possible regulatory link between the PTS and the tricarboxylic acid TCA cycle (124) (see also the section on interaction between acetate kinase and EI, below). As discussed above, an amino-terminal domain of EI containing the active-site His residue (His-189 in *E. coli*) participates in reversible phospho exchange with HPr, while a carboxy-terminal domain appears to be necessary for interaction with PEP and dimerization (256).

Phospho(His)-HPr, the product of the EI-catalyzed reaction, has been shown to phosphorylate all EIIA domains or proteins in which these reactions have been studied (reaction 3). The phospho group is subsequently transferred to the carbohydrate substrate, as discussed in the next section. Phosphorylation of His-15 at the N-1 position in *E. coli* HPr may be favored because the N-3 atom of this residue is apparently protonated at physiological pH values and is a hydrogen bond donor, possibly to a carboxylate group, in unphosphorylated HPr (497). This would make the N-1 atom a better nucleophile for the phospho group in P-EI. On phosphorylation of HPr, the hydrogen bond involving the N-3 atom breaks, indicating a conformational change in the protein (497).

The phosphoryl transfer potential ($\Delta G^{\circ\prime}$ for hydrolysis) of P-HPr from *S. typhimurium* is −13 kcal/mol (ca. −54 kJ/mol), still close to that for the original phospho donor, PEP (523). As discussed below, at least some circumstantial evidence suggests that P-HPr may interact with, or phosphorylate, other cellular proteins involved in chemotaxis and metabolic regulation, which may explain the fact that it retains most of the phosphoryl transfer potential of PEP. Recently, phospho exchange between P-HPr and free HPr has been observed in highly purified preparations of *E. coli* HPr (498), but the significance of this reaction with regard to HPr structure and function remains to be determined.

As discussed above, three-dimensional structural studies of HPr suggest an essential role of Arg-17 in HPr function, possibly involving stabilization of P-(His-15)-HPr by interaction of Arg-17 with the phospho group (172). The functional roles of amino acyl residues of HPr have also been

studied recently by site-directed mutagenesis of *ptsH*. As expected, mutation of His-15 to Ala resulted in a protein that was unable to be phosphorylated by EI and PEP (387). Consistent with the structural studies, mutation of Arg-17 to either Gly or Lys in *E. coli* HPr resulted in a protein which was essentially inactive in participating in the overall PEP-dependent phosphorylation of mannose, whereas mutation of 33 other residues revealed no essential roles for any of these residues in phosphotransfer (449). From the crystal structures of HPr and the IIA^{Glc} domain of *B. subtilis*, a role for Arg-17 in phosphotransfer from P-HPr to the IIA domains of EIIs has also been proposed (172, 254). It is hypothesized that Arg-17 switches from a salt bridge with the phospho group in P-HPr to a salt bridge with two Asp residues near the active-site His of IIA^{Glc}, thus destabilizing P-HPr and facilitating phosphotransfer to His-83 of *B. subtilis* IIA^{Glc}.

Recently, support for this phosphotransfer mechanism was obtained with molecular modeling studies using the crystal structures of these two proteins (171). It was found that HPr and IIA^{Glc} from *B. subtilis* contained complementary surface topographies, and in the modeled binary complex the phospho group of P-HPr would be buried at the interface between the two proteins. No large conformational changes in either protein were necessary for this interaction. Moreover, in the modeled complex between free HPr and IIA^{Glc}, Arg-17 of HPr was in an ideal position to form the proposed salt bridges with Asp-31 and Asp-87 of IIA^{Glc} that are hypothesized to facilitate phosphotransfer to IIA^{Glc} (171).

Phosphotransfer Mechanisms of EIIs

EIIA proteins. As mentioned above, a His residue is also the phospho acceptor from P-HPr in all IIA proteins or domains studied to date. Of the separate IIA proteins, IIA^{Glc} has been studied in the most detail. As in EI and HPr, the phosphoryl transfer potential of P-IIA^{Glc} is also near that of PEP ($\Delta G^{O\prime}$ for hydrolysis is ca. −14 kcal/mol [ca. −59 kJ/mol] [523]). Although P-IIA^{Glc} has not been shown to phosphorylate any proteins other than PTS IIBC and IICB complexes, IIA^{Glc} does perform critical roles in general transport regulation, as discussed in the section on regulation by the PTS (below).

The extreme amino terminus of IIA^{Glc} appears to be important for its interaction with and phosphorylation of $IICB^{Glc}$. A variant of IIA^{Glc}, which migrates faster on nondenaturing gels than native IIA^{Glc} and thus was designated IIA^{Glc}_{Fast}, is found in most preparations of this protein and was shown to be derived from the "slow" form (IIA^{Glc}_{Slow}) by proteolytic cleavage of the first 7 amino-terminal residues (283, 286). IIA^{Glc}_{Fast} is phosphorylated by P-HPr at a rate similar to that seen for IIA^{Glc}_{Slow}, but P-IIA^{Glc}_{Fast} donates its phospho group to $IICB^{Glc}$ at only 2 to 3% of the rate seen with P-IIA^{Glc}_{Slow} (283). Moreover, a derivative of IIA^{Glc}_{Slow} that was chemically modified on the amino-terminal Gly residue (183) had properties similar to IIA^{Glc}_{Fast}, whereas a mutant IIA^{Glc} in which Phe-5 was replaced by Trp was actually more efficient than the wild-type protein in in vitro phosphorylation of αMG (365). Whether the two electrophoretic forms of IIA^{Glc} have any physiological significance, e.g., in regulation of glucose PTS activity, remains to be determined.

Detailed kinetic studies of the interactions of HPrs and IIA domains or proteins from the same or different organisms have been reported recently (388). For the IIA^{Glc} protein from *E. coli*, the apparent K_m in carbohydrate phosphorylation assays for *E. coli* P-HPr was 0.3 μM, but that for *B. subtilis* P-HPr was 100 μM. For the IIA^{Glc} domain from *B. subtilis*, the K_m values for *B. subtilis* P-HPr and *E. coli* P-HPr were 0.5 and 0.1 μM, respectively. Finally, for the IIA^{Mtl} protein from *B. subtilis* the apparent K_m in mannitol phosphorylation for *B. subtilis* P-HPr was 0.6 μM (388), whereas for $IICBA^{Mtl}$ from *E. coli* a K_m for P-HPr of about 1 μM has been reported (403). Most of these values are remarkably similar and also emphasize the ability of single HPr species to recognize IIA domains or proteins of quite different primary structure as mentioned above.

Site-directed mutagenesis studies (365, 388) coupled with the recent three-dimensional structure determinations of IIA^{Glc} from *B. subtilis* (254) and *E. coli* (540) have provided clues to structure-function relationships in the phosphotransfer activities of these proteins. As expected, replacement of the active-site His-90 in IIA^{Glc} from *E. coli* with Gln (365) or of the corresponding residue (His-83) in *B. subtilis* IIA^{Glc} with Ala (388) resulted in inactive proteins that could not be phosphorylated by P-HPr. If the same substitutions were made with the nearby His-75 in the *E. coli* protein (365) and the corresponding His-68 in *B. subtilis* IIA^{Glc} (388), the mutant proteins could still be phosphorylated by P-HPr but could not transfer the phospho group to $IICB^{Glc}$. Thus, these latter His residues may have some important role in phosphotransfer from P-IIA^{Glc} to $IICB^{Glc}$. Although several possibilities for this role have been presented (254, 365, 540), the exact function of this His residue in phosphotransfer remains to be determined.

$EIICB^{Glc}$. As mentioned above, purified $IICB^{Glc}$ catalyzes PEP-dependent phosphorylation of glucose in the presence of EI, HPr and IIA^{Glc}. It also catalyzes phospho exchange between P-IIA^{Glc} and IIA^{Glc} and between glucose 6-phosphate and glucose, indicative of reactions 4 and 5 (above), respectively (105, 389). The first evidence that $IICB^{Glc}$ is phosphorylated as a catalytically important intermediate was obtained by examination of the stereochemical course of the phosphotransfer reactions carried out by the *E. coli* glucose PTS. Enzyme-catalyzed phosphotransfer reactions generally proceed with inversion of the stereochemical configuration of the substituents attached to the phosphorus atom for each phosphotransfer step (216). The stereochemical course can be directly measured if the phospho substrate contains a chiral phospho group in which two of the oxygen atoms are separately enriched in ^{17}O and ^{18}O. The stereochemical configuration of these groups around the phosphorus atom (*R* or *S*) can be determined in both the substrate and the product of the reaction by ^{31}P-NMR techniques. By using chiral PEP, it was shown that phosphotransfer to αMG, catalyzed by EI, HPr, IIA^{Glc}, and membranes containing $IICB^{Glc}$, proceeded with overall inversion of the configuration about the phosphorus in the αMG 6-phosphate product compared with PEP (15). This implied an odd number of phosphotransfer steps in the overall reaction (reactions 1 to 5, above). Since the participation of covalent phospho intermediates of EI, HPr, and IIA^{Glc} was well established, these results implied that there was an even number of phosphotransfer steps between P-IIA^{Glc} and αMG 6-phosphate (presumably two), i.e., that a catalytically important P-$IICB^{Glc}$ intermediate exists (15).

Subsequently, covalent labeling of $IICB^{Glc}$ with [^{32}P]PEP was demonstrated in membranes in the presence of EI, HPr, and IIA^{Glc} (337). This observation was then extended to purified $IICB^{Glc}$ from *S. typhimurium*, and it was shown that the stoichiometry was 0.6 to 0.8 phospho group incorporated

per $IICB^{Glc}$ polypeptide (99). P-$IICB^{Glc}$ could transfer its phospho group to glucose to form glucose 6-phosphate in the absence of IIA^{Glc}, but the rate of phosphotransfer was greatly stimulated by addition of unphosphorylated IIA^{Glc} (99). These latter results suggest that IIA^{Glc} may allosterically activate the phosphotransfer reaction from P-$IICB^{Glc}$ to glucose.

The kinetics of phosphotransfer to carbohydrate catalyzed by *E. coli* $IICB^{Glc}$ have also been examined. Ping-pong kinetics were observed at constant IIA^{Glc} concentrations and varying concentrations of P-HPr and glucose (293), whereas a sequential kinetic mechanism was inferred from results obtained by varying the concentrations of P-IIA^{Glc} and carbohydrate at constant HPr concentrations (146). The latter result was surprising because of the known existence of a catalytically important P-$IICB^{Glc}$ intermediate, in which case ping-pong kinetics might have been expected. However, these results have been explained by proposing that only the P-$IICB^{Glc}$–IIA^{Glc} complex, and not free P-$IICB^{Glc}$, catalyzes phosphotransfer to carbohydrate efficiently, accounting for the sequential kinetics with P-IIA^{Glc} and carbohydrate as the varied substrates (146, 396). This proposal is supported by the direct determination that this is indeed the case (99). Sequential kinetics that have been observed with other EIIs in which there is a separate IIA protein (146, 266, 267, 453) may also be explained in this manner. Like many PTS EIIs, $IICB^{Glc}$ from *E. coli* or *S. typhimurium* has a high affinity for its substrates in the carbohydrate phosphorylation reaction. The apparent K_m for glucose has been reported to be 3 to 10 μM (293, 471), and that for αMG has been reported to be from 6 to 28 μM (146, 471), whereas that for P-IIA^{Glc} is between 2 and 5 μM (146, 286, 388).

As mentioned above, the residue in $IICB^{Glc}$ that is phosphorylated by P-(His-90)-IIA^{Glc} is Cys-421 (102, 287a). Consistent with this, it was shown that changing Cys-421 to Ser by site-directed mutagenesis resulted in an inactive protein that could not be phosphorylated at all by P-IIA^{Glc} (315).

EII^{Mtl}. Because *E. coli* EII^{Mtl} was the first membrane-bound EII to be purified (187) and the *mtlA* gene was the first such gene to be cloned and sequenced (235), more is known about its structure and mechanism than for any other PTS EII. As discussed above, it combines IIA, IIB, and IIC domains in a single polypeptide and catalyzes PEP-dependent phosphorylation and transport of mannitol in the presence of EI and HPr. Purified II^{Mtl} also catalyzes two phospho-exchange reactions, one between P-HPr and HPr (478) and the other between mannitol 1-phosphate and mannitol (186, 187). These reactions are presumably simply indicative of the reversibility of reactions 3 and 5. Like $IICB^{Glc}$, it can be phosphorylated by [^{32}P]PEP, either in unfractionated membranes (516) or in a purified form (404). Unlike II^{Glc}, however, only the general PTS proteins EI and HPr are required to observe phosphorylation, because IIA^{Mtl} is included as the carboxy-terminal domain in this protein.

As expected, *E. coli* II^{Mtl} gives ping-pong kinetics for mannitol phosphorylation with mannitol and P-HPr as the varied substrates (146, 403), consistent with a catalytically important P-II^{Mtl} intermediate. This intermediate was also observed in kinetic experiments with pyruvate- and mannitol 1-phosphate-burst assays (403). The apparent K_m for mannitol has been reported to be 2 to 11 μM (146, 186, 238, 403), and that for P-HPr has been reported to be 1 to 7 μM (146, 403) for II^{Mtl}. The mechanism and exact kinetic constants for the phospho-exchange reaction between mannitol and mannitol 1-phosphate, which is catalyzed by II^{Mtl} alone, have been difficult to determine because of the strong substrate inhibition observed with both substrates of this reaction (186, 403).

The stoichiometry of phospho-group incorporation into purified II^{Mtl} was found to be nearly two per polypeptide chain (329), and two different phospho peptides were isolated from fully phosphorylated II^{Mtl} (328). Although the phosphorylated amino acid in each of these peptides was not directly identified, properties such as the pH dependence of phospho-group hydrolysis were used to argue strongly that the phosphorylated residues were His-554 and Cys-384. Moreover, the stoichiometry of phospho-group incorporation was reduced to one per polypeptide after treatment with sulfhydryl reagents that react with Cys-384 and also inactivate the protein (327–329). Consistent with these assignments were studies of carboxy-terminal deletion mutants of EII^{Mtl} (147), in which it was shown that small deletions of the carboxy terminus inactivated PEP-dependent phosphorylation but not phospho-exchange activity, which was lost only after deletion of most of the IIB^{Mtl} domain. Moreover, ^{32}P-labeled EII^{Mtl} is labeled only in the hydrophilic, C-terminal half of the protein (469), and the separately expressed IIBA part of the protein, containing His-554 and Cys-384, is covalently phosphorylated by PEP, EI, and HPr in the absence of the IIC domain (527). These observations, as well as results of studies in which the separately expressed IIA^{Mtl} (505) and $IIBA^{Mtl}$ (504, 527) domains were used to complement mutated or truncated II^{Mtl}, demonstrated that His-554 in the IIA domain was most probably the phospho acceptor from P-HPr and that P~Cys-384 (in the IIB domain) was the phospho donor to mannitol. Therefore, there must be phosphotransfer between these residues in intact II^{Mtl}.

Recently, P~His and P~Cys were identified by ^{31}P-NMR in fully phosphorylated II^{Mtl} (326). Furthermore, site-directed mutagenesis studies have shown that when His-554 is changed to Ala (506, 526) or Asp (526), the resulting proteins are inactive in PEP-dependent mannitol phosphorylation but still catalyze mannitol:mannitol 1-phosphate phospho exchange. Neither of these mutant proteins can be phosphorylated at all by PEP in the presence of EI and HPr (526), consistent with the assignment of His-554 as the phospho acceptor from P-HPr. When Cys-384 was changed to Ser (506) or into Asp or His (526), the resultant proteins were inactive in PEP-dependent phosphorylation, but at least the C384D and C384H mutants (526) could be phosphorylated by PEP (at His-554). A role for an additional His residue in II^{Mtl} (His-195), perhaps involving substrate binding and/or phosphorylation, was also inferred on the basis of these mutagenesis studies (526).

Finally, the stereochemical course of mannitol phosphorylation by the mannitol PTS has also been recently determined (301), and the results were fully in accord with the presence of two catalytically important P-EII^{Mtl} intermediates. Taken together, this evidence strongly supports the assignments of His-554 and Cys-384 as the sequential phosphorylation sites in the IIA^{Mtl} and IIB^{Mtl} domains, respectively, as shown in Fig. 3.

EII^{Man}. As mentioned above, II^{Man} differs from most other PTS EIIs in combining the IIA and IIB domains in a single hydrophilic protein and in having two proteins, IIC and IID, making up the integral membrane part of the complex. It also shows little similarity to other EIIs so far sequenced, except for the EII complexes specific for L-sorbose in *K. pneumoniae* and fructose in *B. subtilis* (278, 535) (Table 2). The 35-kDa $IIAB^{Man}$ protein has been purified (107). Its domains can be separated by mild trypsinolysis,

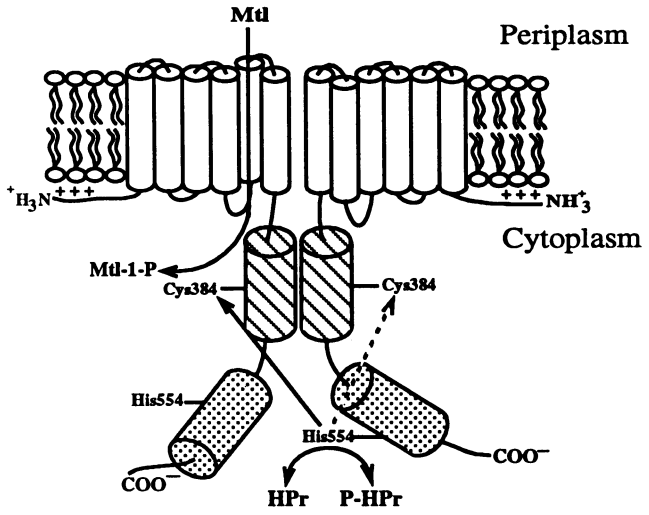

FIG. 3. Topological model of EII^{Mtl} showing the pathway of phosphotransfer from P-HPr to mannitol. The protein is shown as a dimer in the membrane with six transmembrane α-helices in the amino-terminal half (IIC domain) of each monomer. The three-dimensional arrangement of these helices with respect to each other is not known. The extreme amino terminus can potentially form an amphipathic α-helix, but its position in the structure has not been experimentally determined. As shown here, its enrichment of positively charged residues suggests that it could interact with the negatively charged phospholipid head groups at the cytoplasmic surface of the membrane. The carboxy-terminal half of the protein is composed of two hydrophilic, cytoplasmic domains: IIA (stippled) containing His-554, the phosphoacceptor from P-HPr; and IIB (hatched) containing Cys-384, the phospho acceptor from P-(His-554)-II^{Mtl} and the phospho donor to mannitol after its translocation through the IIC domain. Intersubunit phosphotransfer between His-554 and Cys-384, followed by phosphotransfer to mannitol (solid arrows), has been observed in mutant heterodimers and presumably also occurs in the native protein. Intrasubunit phosphotransfer between these two residues (dashed arrow) has not yet been directly shown but has been inferred from experiments suggesting that the monomer possesses some PEP-dependent mannitol phosphorylation activity. The putative cytoplasmic loop between helices 4 and 5 may have roles in mannitol binding and/or phosphotransfer. See the text for details and references. Modified with permission from Fig. 4 of reference 526.

and each domain has been separately expressed by subcloning the appropriate parts of the *manX* (*ptsL*) gene (106). The IIA domain (13 kDa) is the amino-terminal part of the protein and is phosphorylated by PEP in the presence of EI and HPr. The phosphorylated residue of the IIA domain was identified chromatographically as His-10 (phosphorylated at the N-3 position). The carboxy-terminal IIB domain (20 kDa) was phosphorylated by PEP only in the presence of EI, HPr, and catalytic amounts of IIA^{Man}. In IIB^{Man}, the phosphorylated residue is His-175 of $IIAB^{Man}$ (at the N-1 position). No phosphorylation of the IIC or IID domains was detected in these experiments (106). When the IIA^{Man} and the IIB^{Man} domains were expressed in *E. coli* as individual polypeptides, the in vitro and in vivo activity was almost similar to that of $IIAB^{Man}$. Increasing or decreasing the length of the linker between the IIA and IIB domain always resulted in an active protein, although its activity was sometimes decreased (106).

These results showed that His-10 of $IIAB^{Man}$ is the phospho acceptor from P-HPr and that it transfers the phospho group to His-175 of the same protein. Moreover, in the presence of the IIC and IID proteins, phospho-exchange activity between glucose 6-phosphate and 2-deoxyglucose (2DG) was observed with IIB^{Man} but not with IIA^{Man} (106). Thus, P~His-175 in the IIB domain could be the phospho donor to carbohydrate (reaction 5). It is therefore possible that in this class of EIIs, P~His is the phospho donor to the carbohydrate, whereas in the other EII classes thus far examined, this role is carried out by P~Cys.

Other EIIs. In contrast to the enteric $IICB^{Glc}$, II^{Mtl}, and II^{Man} proteins, there is less direct information about structure-function relationships in most other EIIs, apart from inferences based on sequence similarities to these three proteins. However, a fairly extensive characterization of site-specific mutant proteins has recently been carried out on $IIBCA^{Bgl}$ of *E. coli* (441). The results of this study were consistent with His-547 in the IIA domain being the phospho acceptor from P-HPr. Asp-551 and Arg-625 appeared to be important in phosphotransfer either from P-HPr to His-547 or from P~His-547 to the IIB domain phosphorylation site. Mutation of Cys-24 and His-306 suggested that either could be the presumed second phosphorylation site in II^{Bgl}, since both appeared to be essential in phosphotransfer to carbohydrate. Finally, a possible role for His-183 in II^{Bgl} was inferred because mutation of this residue to Arg affected both the substrate specificity of the protein and its ability to catalyze both PEP- and carbohydrate phosphate-dependent phosphorylation (441).

Potential phosphorylation sites have also recently been investigated in $IICB^{Lac}$ proteins in gram-positive bacteria. All four His and all seven Cys residues in $IICB^{Lac}$ from *L. casei* were each individually replaced by site-directed mutagenesis (3). Of these, only Cys-483 was essential for lactose phosphorylation activity. Similar studies of $IICB^{Lac}$ from *S. aureus* also concluded that the homologous Cys-476 of this protein was the phosphorylation site (115). Thus, in these EIIs as well, Cys appears to be the phospho acceptor from the IIA protein and the phospho donor to carbohydrate.

Finally, II^{Fru} from *Rhodopseudomonas sphaeroides* also appears to have at least one activity-linked cysteine, although the phosphorylation site has not yet been determined. Interestingly, this protein appears to bind Zn^{2+}, and the binding of this cation protects against thiol oxidation and concomitant inactivation (268). The role if any, of this Zn^{2+}-binding site in II^{Fru} remains to be determined.

Oligomerization and evidence for intersubunit phosphotransfer. The oligomeric structure of II^{Mtl} has been the most extensively studied. Considerable evidence suggests that both PEP-dependent and phospho-exchange activities of this protein are optimally catalyzed by an oligomeric form of this protein, minimally a dimer (184, 209, 251, 264, 325, 393, 404, 405, 468). This raises the question of whether phosphotransfer between the P-(His-554)-IIA^{Mtl} domain and Cys-384 in the IIB domain occurs within a single polypeptide in the oligomer or between these two residues in different oligomeric subunits. Intersubunit phosphotransfer between these residues was first observed in vitro in detergent solution by using a wild-type II^{Mtl} protein in which Cys-384 had been inactivated by N-ethylmaleimide, and a carboxy-terminally truncated II^{Mtl} that lacked His-554 but still retained Cys-384. The truncated II^{Mtl} could be phosphorylated by PEP, EI, and HPr in the presence of *N*-ethylmaleimide-treated wild-type protein but not in its absence. Moreover, the phosphorylated, truncated protein could then transfer its phospho group to mannitol (469).

More recently, this question has been addressed by using site-directed mutants of II^{Mtl} at each of the two phosphory-

lation sites. The H554A and C384S mutant proteins, each of which was inactive alone in PEP-dependent mannitol phosphorylation, could catalyze this reaction when present together in detergent solution (506). This observation was extended to the membrane-bound, in vivo situation by expressing the inactive mutant proteins H554A (or H554D) and C384H in the same (*ΔmtlA*) cell. Such cells were positive for mannitol fermentation and phosphorylated mannitol at a rate that was 40% that of cells expressing a similar amount of wild-type protein (526). These experiments showed that intersubunit phosphotransfer between His-554 and Cys-384 can occur and could explain the requirement for an oligomer of II^{Mtl}, at least in its PEP-dependent activity. However, there is evidence that a dissociated form of II^{Mtl}, observed at low concentrations of the purified protein, can also catalyze PEP-dependent phosphorylation but with a specific activity that was about 25% of that of the oligomer (264). Therefore, the possibility of intrasubunit phosphotransfer cannot yet be ruled out for II^{Mtl} (Fig. 3).

For $IICB^{Glc}$, it was first postulated that this protein was active as a dimer (99), but later experiments suggested that it could catalyze at least phospho exchange between glucose 6-phosphate and glucose as a monomer (288). However, it has also been shown that $IICBA^{Nag}$ or $IIBCA^{Bgl}$ can complement $IICB^{Glc}$ or $IIBC^{Scr}$ in an *E. coli* strain that is lacking IIA^{Glc} in glucose and αMG transport and phosphorylation (355, 441, 507). Undoubtedly, this is because the IIA domains of both of these membrane-bound proteins are highly similar in primary structure to IIA^{Glc}, as discussed above. Indeed, it was previously shown that a truncated II^{Nag} from *K. pneumoniae*, lacking its IIA domain, could be complemented in vivo by IIA^{Glc} (509). Therefore, a functional heterodimer must at least transiently form in the membrane between II^{Nag} or II^{Bgl} and $IICB^{Glc}$, and it is probable that intersubunit phosphotransfer occurs in these cases as well.

Even if $IICB^{Glc}$ is active as a monomer, this does not necessarily indicate a fundamental difference in mechanism compared with II^{Mtl}. In II^{Mtl} the IIA domain is covalently linked to the IIB and IIC domains on the same polypeptide. Thus, the IIA domain may be physically restricted from phosphorylating the IIB domain in the same polypeptide at a high rate but may be able to phosphorylate Cys-384 in a neighboring subunit of the oligomer more easily. In II^{Glc}, the IIA domain is a separate, soluble protein and thus should be able to phosphorylate the IIB domain of a IICB glucose monomer.

Finally, the relationship of oligomerization to phosphotransfer reactions in II^{Man} has also been investigated. $IIAB^{Man}$ appears to exist as a very stable dimer (107). It was subsequently shown that P-$IIAB^{Man}$, phosphorylated at both sites, could phosphorylate either the isolated IIA or the isolated IIB domain (106). This observation suggests that intermolecular phosphotransfer between these two domains in a native $IIAB^{Man}$ dimer can also occur, although this possibility has not yet been directly tested (e.g., by site-directed mutagenesis).

Mechanism of Transport Catalyzed by EIIs

Although much has been learned about the phosphorylation mechanisms of a number of the EIIs by using the (primarily) in vitro studies summarized above, studies designed to determine the mechanism of translocation catalyzed by the EIIs must, by definition, also include experiments with whole cells or at least with membrane vesicles of known orientation. This is illustrated by comparisons of the apparent K_ms for PEP-dependent phosphorylation, determined in vitro, with those for uptake of PTS substrates. For example, in a study with *S. typhimurium* (471), the K_m for αMG in PEP-dependent phosphorylation catalyzed by $IICB^{Glc}$ was reported to be 6 μM but the K_m for αMG uptake by whole cells was 170 μM. Conversely, the K_m for mannitol uptake by $IICBA^{Mtl}$ in *E. coli* was reported to be about 0.4 μM (238) but the K_m for phosphorylation in vitro is about an order of magnitude higher, as discussed above. Uptake includes binding to a periplasm-facing site, followed by translocation and phosphorylation. However, EIIs can apparently catalyze nonvectorial phosphorylation without translocation, as discussed below, so that kinetic and mechanistic studies of phosphorylation alone are insufficient to characterize the entire translocation mechanism.

It has often been assumed in the past that transport and phosphorylation of carbohydrates by the PTS are simultaneous events. However, in principle, transport of a PTS substrate through the membrane could either be directly coupled to phosphorylation of the substrate or be the consequence of phosphorylation of the EII followed by phosphorylation of the carbohydrate in a separate step on the inside surface of the membrane. As we shall see, current evidence favors the latter mechanism, in which it appears that only the phosphorylated forms of the EIIs carry out transport of their natural substrates at a high rate. This transport probably occurs by facilitated diffusion (net movement of the substrate down its concentration gradient) rather than by an active mechanism requiring an additional input of energy other than PEP. In this section, we shall therefore review studies designed to understand the translocation mechanism, focusing on II^{Mtl} and II^{Glc}, for which the most information is known.

EII^{Mtl}. Both purified and membrane-bound II^{Mtl} from *E. coli* bind mannitol with high affinity. With the purified protein, one high-affinity binding site per dimer (K_D = 0.1 μM) and a lower-affinity site (K_D = ca. 9 μM) were found (329). For the membrane-bound protein, both high- and lower-affinity binding sites for mannitol have been found with K_Ds of about 40 nM (262) and 1 μM (36, 147). Binding is apparently independent of the carboxy-terminal half of the protein and both phosphorylation sites therein, since both truncated II^{Mtl}, lacking His-554 and Cys-384 (36, 147, 262), and mutant proteins in which His-554 has been replaced (526) still bind mannitol with affinities and stoichiometries similar to those of the wild-type protein. Moreover, although the binding affinity for mannitol is decreased somewhat in a mutant defective in the second phosphorylation site (C384S), phosphorylation of this protein at His-554 had no further effect on binding (263).

From the predicted secondary structure of II^{Mtl} in the membrane (474), it might be reasonable to propose that at least some of the 20 polar residues (or those capable of hydrogen bonding) that are within the membrane could form at least part of a mannitol-binding site. Indeed, only the IIC domain is necessary for mannitol binding (147, 262), but deletion of as little as the last putative transmembrane helix destroys detectable binding activity (36). However, there is as yet no evidence that any of these intramembrane residues is specifically involved in forming the binding site.

A mutation in II^{Mtl}, G253E, has been isolated, and the resulting protein was shown to be able to phosphorylate but not transport mannitol (273). This residue is found in a putative cytoplasmic loop of II^{Mtl} between transmembrane helices 4 and 5 (474). The additional observation that His-195, also present in this loop, appears to have an important

role in mannitol binding and/or phosphorylation (526) suggests that this portion of the protein may interact with the substrate and also may be involved in translocation. Thus, if this region is not itself transmembrane, it is possible that it projects up into a channel formed by the transmembrane helices (242) or, alternatively, serves as a "cap" or "gate" for such a channel.

The kinetics of mannitol binding, translocation and phosphorylation by II^{Mtl} have recently been studied in detail by using both inside-out (ISO) and right-side-out (RSO) vesicle systems (261, 262, 265) and purified II^{Mtl} reconstituted into proteoliposomes (92). Unphosphorylated II^{Mtl} can apparently catalyze facilitated diffusion of mannitol very slowly, and this process can be carried out by the IIC domain alone (92, 262). Phosphorylation of II^{Mtl} increases the translocation rate by several orders of magnitude (92, 265), but translocation in this case is not strictly coupled to the transfer of the phospho group to mannitol to form mannitol 1-phosphate. Somewhat less than 50% of the mannitol translocated by the phosphorylated protein in ISO vesicles was phosphorylated before it dissociated from the protein as measured by a mannitol 1-phosphate burst "trapping" assay. These mannitol molecules, however, could apparently rebind to II^{Mtl} at a cytoplasm-facing binding site and subsequently be phosphorylated by the protein (265). In accord with the previously observed higher phosphorylation K_m for mannitol compared with the K_m for uptake, the uptake K_m in the reconstituted liposome system was found to be <10 μM, whereas the K_m for phosphorylation (without transport) was about 66 μM (92).

These results, taken together, suggest that the loaded, periplasm-facing mannitol-binding site of II^{Mtl} converts to a cytoplasm-facing orientation only slowly in unphosphorylated II^{Mtl}, that phosphorylation of the protein greatly increases this translocation, and that phosphorylation of mannitol by P-II^{Mtl} is not strictly coupled, mechanistically, to conversion (i.e., the protein can carry out facilitated diffusion). Most recently, kinetic evidence has also been presented suggesting that the "inward-facing" and "outward-facing" states of the protein are related through a state in which the mannitol-binding site is occluded (261). In that study, it was also suggested but not proven that in a II^{Mtl} dimer, each binding site was oppositely oriented and that reorientation of one site was accompanied by reorientation of the other in the opposite direction. If the sites in each of these different orientations have a different affinity for mannitol, this could explain many of the binding and kinetic data (e.g., the high- and low-affinity sites observed with the purified protein and the different K_ms for transport and nonvectorial phosphorylation). Although this is an attractive model, further work is necessary to test its validity.

The kinetic data are consistent with the mutant studies described above. The ability to isolate "uncoupled" mutants of II^{Mtl} which can phosphorylate without significant transport (273) argues that these processes are not likely to be obligatorily coupled even in the wild-type protein. In accord with this, it has been found that even wild-type II^{Mtl}, and the wild-type IIC^{Mtl} domain alone, can carry out facilitated diffusion of D-arabinitol (248), a low-affinity substrate of II^{Mtl} for both transport and phosphorylation (190, 237). Moreover, in tight *ptsI* mutants of *E. coli*, induction of either the *mtl* or *gut* operons is still possible but only if the EII specific for the corresponding inducer is present (247, 459), indicating that the EIIs can catalyze facilitated diffusion without phosphorylation. It should be realized, however, that the rate of solute transport required for induction is much lower than that required for metabolism. *ptsI* mutants are unable to grow on PTS carbohydrates, even if subsequent intracellular metabolism of the nonphosphorylated carbohydrates is possible (361). As discussed below, the conclusion that at least some EIIs can carry out facilitated diffusion under certain conditions also offers an explanation for the in vivo phenomena of inducer expulsion and intracellular phosphorylation of PTS substrates apparently catalyzed by at least some EIIs.

Finally, it is clear that the events leading to translocation of a PTS substrate by the EIIs must involve a cycle of conformational changes in these proteins. For EII^{Mtl}, conformational changes resulting from substrate binding (209, 468), from phosphorylation of the protein (209, 265, 499) and from interactions of the various domains of the protein (263) have been observed from kinetic and physicochemical measurements. Further studies are necessary, however, to determine the exact nature of these conformational changes and their relationship to translocation and phosphorylation catalyzed by II^{Mtl}.

EII^{Glc}. Although less is known about the kinetic properties of carbohydrate binding and translocation by $IICB^{Glc}$ than by II^{Mtl}, recent molecular genetic studies support similar mechanisms for these two proteins. In particular, it was shown that a hybrid protein, constructed by fusing the IIC^{Glc} domain to the IIB^{Nag} domain, both from *E. coli*, was specific for glucose, not *N*-acetylglucosamine (179). Therefore, as for II^{Mtl}, the carbohydrate binding/translocation site must reside in the IIC^{Glc} domain. Also as for II^{Mtl}, this binding site has a high affinity for glucose (K_D = 1.5 μM) (414). Moreover, mutants of *S. typhimurium* were selected in which II^{Glc} could carry out facilitated diffusion, but not phosphorylation, of glucose (351, 413). These mutants had a transport K_m for glucose that was about 1,000-fold higher than with the wild-type protein, and they could be secondarily mutated in glucose-limited chemostat cultures to proteins with intermediate affinities for glucose (411).

Recently, II^{Glc} mutants of *E. coli* that also uncouple transport from phosphorylation were isolated by using the plasmid-encoded *ptsG* gene (414). These mutant proteins were able to transport glucose in cells lacking EI and HPr and had K_m values for glucose oxidation (0.5 to 2.5 mM) in these cells that were about 100-fold higher than that for the wild-type protein. However, they were also able to catalyze PEP-dependent phosphorylation of αMG, and in a pts^+ background, some of these mutants transported glucose at a higher rate and with a lower apparent K_m than in the *ptsHI* mutant in which they were isolated. A number of these mutant *ptsG* genes were sequenced, and four different mutations, R203S, V206A, K257N and I296N, were found that led to this general phenotype (414). According to a topology model of II^{Glc} (105), all these positions in the primary sequence lie within a hydrophilic domain that may be cytoplasmic. One of these mutations (I296N) lies within a sequence (GITE) that is highly conserved in many EIIs (248). Thus, as in II^{Mtl}, at least part of the glucose-binding/translocation site may not reside within the transmembrane regions of the protein.

By growing *S. typhimurium* anaerobically in a chemostat, it could be shown that the transport of glucose via an uncoupled II^{Glc} does not require energy. The molar growth yields on glucose (Y_{Glc}) were the same in a strain in which glucose is transported and phosphorylated via the PTS and in an uncoupled *ptsG* mutant in which glucose is transported via an uncoupled II^{Glc} followed by ATP-dependent glucose phosphorylation via glucokinase (413).

It is interesting that all mutations in II^{Glc} which have been

characterized and which allow efficient facilitated diffusion of the substrate also significantly increase the K_m for transport. In addition, as mentioned above, a low-affinity substrate of II^{Mtl}, D-arabinitol, is also apparently transported by facilitated diffusion. Moreover, facilitated diffusion of galactose, a poor substrate of II^{Glc} and II^{Man}, has been reported to be carried out by II^{Glc} in *E. coli* (223) and by II^{Man} in *S. typhimurium* (350). Similarly, trehalose can be taken up with low affinity by facilitated diffusion via II^{Man} in *S. typhimurium* (357). Possibly, low-affinity substrates of wild-type or mutant EIIs cannot tightly lock the enzyme into the state in which facilitated diffusion is slow (e.g., the occluded state that has been observed for unphosphorylated II^{Mtl} [261]), and facilitated diffusion is therefore more rapid in these cases.

Conversely, mutations have been isolated in II^{Glc}, like in II^{Mtl}, that preferentially affect transport compared with phosphorylation (37). These *E. coli* mutants were selected on the basis of resistance to externally added αMG (which is toxic if transported and phosphorylated by II^{Glc}) coupled with the ability to still phosphorylate intracellular glucose. In general, these mutant proteins had a severely impaired ability to transport αMG while retaining significant PEP-dependent phosphorylation activity for both glucose and αMG. They cluster in three hydrophilic areas of the protein: the amino-terminal region (two different mutations of Met-17), which is postulated to be cytoplasmic, and two other regions (mutations at positions 149, 150, 157, 339, and 343) that are presumed to be periplasmic loops (37). Therefore the periplasmic mutations either could define part of an extracellular binding site or could be involved in a conformational change related to translocation of the substrate. Interestingly, the amino acid sequence immediately following Met-17 in II^{Glc} shows some similarity to a region in the melibiose carrier believed to be involved in carbohydrate binding (37). In this regard, it is also worth noting that a mutation in the amino-terminal region of II^{Bgl} of *E. coli* (C24S) has been shown to uncouple glucose transport from phosphorylation catalyzed by this protein (441).

Finally, phosphorylation by II^{Glc} of glucose generated intracellularly in *E. coli* from maltose has also been observed (315). This demonstrates that even the wild-type protein can carry out phosphorylation from within, without transport, at least under these physiological conditions. In this respect, then, II^{Glc} also resembles II^{Mtl}, although for the latter protein, nonvectorial phosphorylation has to date been convincingly demonstrated only in vesicular systems, as discussed in the previous section.

Consensus model for translocation catalyzed by EIIs. From the foregoing discussion, it is reasonable to propose the following sequence of events leading to the transport and phosphorylation of a PTS carbohydrate, at least for II^{Mtl} and II^{Glc}.

(i) The periplasmic substrate binds with high affinity to its specific EII. If the EII is not in its phosphorylated form (and, for $IICB^{Glc}$, is also not complexed with the IIA protein), the substrate is only slowly translocated by facilitated diffusion to a cytoplasm-facing orientation, if at all.

(ii) Phosphorylation at the IIB site (a Cys residue in II^{Mtl} and II^{Glc}) allows rapid translocation of the substrate to a cytoplasm-facing orientation (i.e., facilitated diffusion occurs via a conformational change in the protein).

(iii) Phosphorylation of the bound substrate by the P-IIB domain then occurs, followed by dissociation of the phospho carbohydrate into the cytoplasm. For II^{Mtl}, at least, the substrate can also dissociate without phosphorylation with about equal probability but can rebind at the cytoplasm-facing site followed by phosphorylation.

Thus, under normal physiological conditions, transport appears to be coupled to phosphorylation (nearly every molecule that is transported is phosphorylated unless there is a fast competing reaction involving the unphosphorylated substrate) but mechanistically the two processes are separate.

Although this overall mechanism agrees with much of the more recent evidence, as discussed in the previous section, it is certainly oversimplified and a number of important questions remain to be answered. For example, as previously reviewed by us (358) and others (284, 396), there are conflicting reports concerning whether PTS substrates generated on the inside of intact cells can be phosphorylated in situ by various EIIs, as would be predicted by the above model, without the substrate first leaving the cell. Although it is not yet possible to reconcile all of the data, it should be recognized that much (but certainly not all) of the evidence for phosphorylation without transport has been obtained with membrane vesicle systems or with mutant EIIs as described above. It is entirely possible that soluble factors or proteins in the cytoplasm inhibit phosphorylation from within in vivo and that this is also influenced by the physiological conditions and the specific EII being studied.

Perhaps the most important unanswered question regarding the mechanisms of the EIIs, however, is exactly how the carbohydrate substrate is translocated from outside to inside through the protein. A complete answer to this question awaits, among other things, a three-dimensional structure determination of a well-studied EII such as II^{Mtl} or II^{Glc}. This may be aided, at least for II^{Mtl}, by the recent development of an overexpression system for this protein (503). Since phosphorylation of the IIB domain, not of the carbohydrate substrate, seems to be tightly coupled to translocation, it is reasonable to presume that it is this phosphorylation event that allows for transport via a conformational change in the protein and that accumulation of PTS substrates is therefore effected by their subsequent phosphorylation by the P-IIB domain, thus trapping them within the cell. A question related to this is whether each EII has a single substrate-binding site that can face either the periplasm or the cytoplasm or whether there might be two different binding sites, one periplasmic and one cytoplasmic, connected by a hydrophilic channel (396). Although this question cannot yet be answered with certainty, the available kinetic and binding evidence favors the single-site, alternative-orientation model (262, 265, 396). It is also still unclear whether this binding/translocation site exists entirely within each EII monomer or whether it might be at the interface of subunits that make up an EII oligomer.

The abilities of at least some EIIs to carry out facilitated diffusion could also help to explain the phenomenon of inducer expulsion (as further discussed in the section on inducer expulsion in gram-positive organisms, below). In inducer expulsion, a PTS substrate, accumulated in cells as the phosphorylated derivative, can be expelled from the cell under certain conditions, a process requiring dephosphorylation of the carbohydrate by an intracellular phosphatase and efflux of the free carbohydrate from the cell. At least in some cases, the efflux process appears to require the same EII that is used to accumulate the carbohydrate (161, 377, 386, 477). If phospho-EIIs can catalyze rapid facilitated diffusion, as appears to be the case at least for II^{Mtl}, this implies that PTS substrates that bind to P-EII on the inside of the cell could also be translocated to the outside before

they are phosphorylated by the P-IIB domain (i.e., efflux of the free carbohydrate occurs) (265). However, no direct measurement of such a process has yet been carried out for any EII.

Regulation of PTS Carbohydrate Uptake Catalyzed by EIIs

Although the synthesis of many EIIs is regulated by a variety of mechanisms, as described later in this review, it is clear that the activities of many EIIs must also be regulated somehow, so that uptake and phosphorylation of PTS substrates do not greatly exceed the capacity of the cells to metabolize them under a given set of environmental conditions. Indeed, uptake of PTS substrates by whole cells is rarely linear with time, and in some cases initial uptake rates are difficult to determine (see, e.g., references 246, 260, 455, and 471). This suggests that as a consequence of PTS carbohydrate uptake, the activities of the EIIs are regulated, directly or indirectly, by some type of feedback mechanism. A number of different mechanisms have been postulated to account for this, including energy-dependent efflux of PTS substrates, regulation by the membrane potential, competition of the EIIs for phospho-HPr, and regulation by intracellular phospho compounds. Because each of these has been extensively reviewed elsewhere (284, 358, 380, 385, 396, 417) and their exact contributions to regulation of EII activity in vivo remain unclear, we will only briefly consider these here, with an emphasis on the more recent work.

If direct regulation of EII activity is a physiologically relevant phenomenon, then the flux of carbohydrates through the EIIs might be expected to be a rate-limiting reaction of the PTS. This has recently been observed for II^{Glc} in *E. coli* (412). At initially wild-type levels of II^{Glc}, gradually raising or lowering the amount of II^{Glc} in cells increased or decreased, respectively, the uptake rate of αMG, indicating that the activity of II^{Glc} exerts considerable control over flux through the PTS (also see the section on details of PTS-mediated regulation, below). Although in these experiments II^{Glc} had a low control coefficient over growth and glucose oxidation at high glucose concentrations (412), other experiments have shown that under glucose-limited conditions, the activity of the glucose PTS does limit growth (180). Thus, the EIIs could be reasonable targets for direct regulation of PTS activities in whole cells, at least for II^{Glc}.

Many workers have shown that the steady-state level of accumulation of αMG by *E. coli* cells could be rapidly decreased by addition of an exogenous energy source (97, 149, 150, 169, 170, 173, 174, 531, 532). This decrease was apparently due to the efflux of free αMG from the cells on addition of the energy source, after hydrolysis of αMG 6-phosphate by an intracellular phosphatase, reminiscent of the phenomenon of inducer expulsion in gram-positive bacteria as discussed above and in the section on Inducer expulsion in gram-positive organisms (below). However, the exact nature of the energy requirement for this process and the question whether II^{Glc} is directly involved in the efflux remain to be determined.

It also appears, however, that influx of substrates via the EIIs is controlled by the energy state of the cell. Thus, it was shown that uncouplers of oxidative phosphorylation and anaerobiosis stimulated αMG accumulation in energized whole cells and membrane vesicles of *E. coli*, whereas donors to the electron transport chain such as D-lactate inhibited accumulation (371). Similar results were obtained for PTS-mediated αMG uptake in the gram-positive bacterium *Brochothrix thermosphacta*, in which it was also observed that αMG accumulation was strongly inhibited at membrane potentials greater than about 50 mV (inside negative) (456). Although in these reports initial uptake rates were not strictly measured, it has been shown by using a stopped-flow apparatus that uncouplers of oxidative phosphorylation also stimulate the initial rate of αMG uptake in *S. typhimurium* but have very little effect on the final steady-state level of accumulation (471). The mechanism of this membrane-potential mediated inhibition of II^{Glc} activity also remains obscure. On the basis of the effects of the membrane potential, oxidizing reagents, and thiol reagents on the phosphorylation of αMG by inside-out vesicles of *E. coli*, it was proposed that high membrane potentials might inhibit II^{Glc} activity by promoting oxidation of thiols in the protein, leading to an increase of the K_m for αMG (395). However, subsequent experiments showed that oxidized II^{Glc}, as well as II^{Mtl}, was essentially inactive (144, 145). Therefore, if the membrane potential inhibits EII activity by sulfhydryl oxidation in vivo, which has yet to be directly shown, it must do so by affecting the V_{max}, and not the K_m, of uptake.

A second mechanism to account for regulation of EII activity by the membrane potential has also been suggested (101, 434). It was proposed that the proton abstracted from the carbohydrate on its phosphorylation might be obligatorily released on the periplasmic side of the membrane as a consequence of the transport mechanism of the EII, thus explaining the inhibition of EII activity by the energized state of the membrane. If this were the case, the EII would be an electrogenic proton pump (434). Such a mechanism could also conserve more of the chemical energy of PEP, in the form of an electrochemical proton gradient, which would otherwise be "lost" in the mere transport and phosphorylation of a carbohydrate by the PTS (101). It also could explain energy-dependent efflux of free PTS carbohydrates, as described above, since an energized state of the membrane would tend to inhibit phosphorylation of PTS substrates and favor facilitated diffusion through the phosphorylated EII. Although this is an intriguing proposal, it has, to our knowledge, not yet been tested experimentally.

As discussed above, various IIA proteins or domains present in the same organism have different K_m values for phosphorylation by P-HPr. This could therefore lead to preferential utilization of one PTS carbohydrate over another in cells expressing the EIIs for both substrates. Such hierarchical utilization of PTS carbohydrates has, in fact, been observed in both gram-negative (6, 96, 220, 243, 246, 444) and gram-positive (79, 86, 388, 489) organisms, but it is not clear that in all cases this is due exclusively to competition for P-HPr. Discrimination between PTS substrates by a single EII which recognizes more than one carbohydrate with different affinities (Table 2) is undoubtedly also a mechanism for preferential PTS-carbohydrate uptake. This has been extensively reviewed elsewhere (358, 385) and will not be further considered here.

Control of EII activity by carbohydrate-phosphates and other intracellular metabolites has also been invoked as a potential feedback control mechanism. Numerous experiments have suggested that intracellular carbohydrate-phosphate products of the PTS can inhibit, directly or indirectly, various EIIs (48, 126, 202, 220, 246, 425). In another study, RSO vesicles from *S. typhimurium* were preloaded with PEP and the initial rate of αMG uptake was measured (259). After an initial high uptake rate, a lower rate of uptake was observed when the intravesicular concentration of αMG

6-phosphate reached ca. 0.2 mM. In contrast, when the vesicles were permeabilized with toluene so that they became leaky to small molecules, only the higher rate (measured as αMG phosphorylation) was observed. These results could indicate that intravesicular αMG 6-phosphate directly inhibits II^{Glc}. However, in toluenized vesicles, inhibition of the phosphorylation activity of II^{Glc} could be observed only at concentrations of αMG 6-phosphate much higher than 0.5 mM, suggesting that this mechanism may be incorrect (259). On the other hand, since we now know that carbohydrate phosphorylation is probably not directly coupled to transport, it is still possible that the phosphorylated carbohydrate products of the EIIs could feedback inhibit transport at lower concentrations than they inhibit phosphorylation. This could be done through direct competition for a substrate-binding site (i.e., product inhibition as mentioned above for the phospho-exchange reaction) or through an as yet uncharacterized allosteric binding site.

The concentrations of other intracellular metabolites have also been proposed to regulate the PTS at the level of EII-mediated uptake. It has been shown for both gram-negative and gram-positive bacteria that starved cells have relatively high concentrations of P_i, PEP, and 2- and 3-phosphoglycerate, whereas on addition of a PTS substrate such as glucose, the concentrations of these metabolites drop with a concomitant increase of other glycolytic intermediates, predominantly fructose 1,6-bisphosphate (279, 488, 492). As described in the section on inducer expulsion in gram-positive organisms (below), high concentrations of fructose 1,6-bisphosphate favor formation of P-(Ser)-HPr, whereas high concentrations of P_i favor its dephosphorylation in gram-positive bacteria. Since P-(Ser)-HPr is phosphorylated at its His residue by EI much more slowly than is free HPr, the relative concentrations of glycolytic intermediates could control PTS-mediated carbohydrate uptake by a feedback mechanism, at least in gram-positive bacteria (79, 188).

A role for P_i in directly regulating EII activity also has been proposed (184). Concentrations of P_i that are observed in starved cells (ca. 50 mM [279]) have been shown to activate II^{Mtl} from *E. coli* in vitro, at least partly by stabilizing its dimeric form (209, 264, 468). Although the specificity for P_i in this activation has been questioned (264), it is nonetheless possible that the intracellular concentration of P_i has some role in regulating II^{Mtl} activity. In particular, it was suggested that in starved cells, high concentrations of PEP and P_i favor the most active form of II^{Mtl}, "priming" it for rapid mannitol uptake and further induction of the *mtl* operon if the cells should encounter mannitol in the medium. Conversely, in cells rapidly transporting and metabolizing mannitol, II^{Mtl} would be in a less active state, helping to ensure that uptake does not exceed the catabolic capacity of the cell (184). The validity of this proposal and the proposed roles of other intracellular phospho compounds in regulating EII activities, however, remain matters for much additional work.

PTS GENETICS AND GENE REGULATION

As for other complex metabolic systems, the analysis of the multiple functions of the PTS in transport, regulation, and chemotaxis at the molecular level has profited from a combination of biochemical and genetic methods.

As discussed in detail in our previous review (358), mutants which were lacking one or more of the PTS proteins and which were defective in the fermentation of numerous carbohydrates gave the first unambiguous evidence for a direct role of the PTS in transport and phosphorylation of the PTS carbohydrates in bacteria. These mutants also pointed to an indirect involvement of the PTS in the metabolism of many non-PTS carbohydrates. Subsequent mapping of the different mutations revealed that the structural genes *ptsH* and *ptsI* for the general PTS proteins HPr and EI, respectively, were clustered in a *pts* operon. In contrast, the genes for the substrate-specific EIIs, with few exceptions, were clustered in an operon or regulon together with the structural genes for the corresponding metabolic enzymes (Fig. 4). Consequently, Lin (257) proposed to restrict the mnemonic *pts* to the genes coding for the general PTS proteins, to name all genes of a substrate-specific operon/regulon by the same three-letter code, and to give to the different operons/regulons individual names. This nomenclature is now generally accepted (for exceptions, see below), and for the most part a mnemonic related to the main substrate and inducer of each system is used (Table 2).

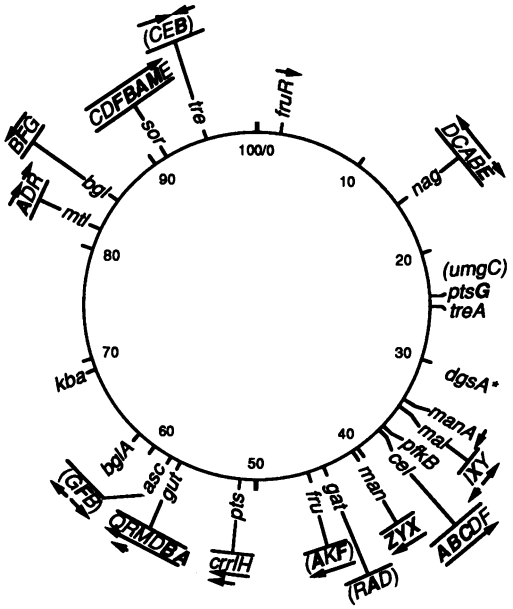

FIG. 4. Simplified genetic map of *E. coli*. The genes involved in uptake and metabolism of various PTS carbohydrates in strains of *E. coli* are shown. Genetic symbols are as in Table 2 and as given by Bachmann (14). Arrows above the operons indicate the direction of transcription. Genes for which this direction relative to neighboring genes is unknown are given in parentheses, and an asterisk (*) indicates that the gene has not been mapped precisely.

General and Specific PTS-Related Operons

The genes for different bacterial PTSs are mostly clustered in operons and regulons together with the genes for the corresponding catabolic enzymes (358). In general, the major substrate is the external inducer. For reasons not fully understood, some PTSs are encoded in a cryptic form in the chromosome and can be expressed only after mutation. Others are encoded only on plasmids or on transposable elements, whereas still others are found on plasmids in one organism and on the chromosome of a closely related organism. We will concentrate in this section on the best-characterized PTS operons, with reference to the others in a tabular form (Table 2).

***pts* operon.** In several enteric bacteria, mutations affecting

the expression of EI, HPr, and IIA^{Glc} have been mapped in a *pts* operon, consisting of the three genes *ptsH*, *ptsI*, and *crr*. For *E. coli*, *S. typhimurium*, and *K. pneumoniae*, mapping these genes relative to each other and to surrounding markers gave the gene order *cysK. . . . .ptsHI crr. . . .cysMA*. The *ptsHI* and *crr* genes, together with the surrounding DNA from these organisms, have been cloned and sequenced by several groups (39, 72, 75, 142, 256, 307, 416, 443). The corresponding gene products have been identified in complementation tests, in various overexpression systems, and by immunochemical methods. According to these data, three major open reading frames (ORFs) are encoded in the *pts* operon. They correspond to the genes *ptsH* (HPr), *ptsI* (EI), and *crr* (IIA^{Glc}). These studies confirmed the near identity of the three genes in all enteric bacteria investigated thus far, including the locations on the gene maps. The *cysK-ptsH* intergenic region is only 385 bp long and lacks an ORF. Downstream from *ptsH* lies *ptsI* followed by *crr*, both with a characteristic ribosome-binding site but no distinct promoter. Further cloning and sequencing data revealed a second promoter, P2, in front of *crr* but located within the 3′ end of *ptsI* (72, 416).

The existence in *E. coli* of a gene, *ptsJ*, which was claimed to map adjacent to *ptsI* (22) was not confirmed by these sequence data. It was shown later that the Mud(Ap *lac*) insertion was in fact located in *ptsH* and oriented in the opposite orientation with respect to *ptsH* (252). Therefore, this type of mutation has the same phenotype as a *ptsI* mutation.

It has been claimed that in *S. typhimurium* a cryptic *pts* operon containing a gene (also unfortunately called *ptsJ*) encoding a second EI (EI*) maps downstream of *crr* (47% cotransduction with *cysA*). Decryptification is allegedly caused by selecting for Pts^+ derivatives in strains thought to be deleted for *ptsHI* (47). Surprisingly, the properties of EI* were identical to those of EI. Until further data become available, it is difficult to evaluate the mutations and genes involved, since cloning experiments thus far have given no evidence for the existence of this operon.

The *ptsH* gene has been cloned and sequenced recently from the gram-positive bacterium *Staphylococcus carnosus* (91, 165). After a 4-bp intergenic space, *ptsI* lies downstream with its ribosome-binding site within the reading frame of *ptsH* (218). Such an arrangement often indicates a tight translational coupling of two adjacent genes in an operon. A similar situation was found in *B. subtilis*, in which the *ptsH* stop codon overlaps the *ptsI* initiation codon by one nucleotide. A single, constitutively expressed *ptsHp* promoter precedes this putative operon. The −10 and −35 boxes are equivalent to those from promoters recognized by the major vegetative sigma factor of *B. subtilis* (142).

Gene loci for substrate-specific PTSs. We will discuss the different genes according to the families described in the previous section and summarized in Fig. 2 and Table 2. We will use this classification to discuss the structure of the corresponding genes and their regulation. It is important to note that, especially in the gram-positive bacteria, biochemically well characterized systems have not always been analyzed thoroughly at the genetic level and vice versa. A few, such as the PTSs specific for dihydroxyacetone (II^{Dha}) (197), D-gluconate (II^{Gnt}) (19), and D-xylitol (II^{Xtl}) (269), have not been well characterized at any level.

(i) Glucose class. The glucose class contains EIIs specific for glucose (Glc), trehalose (Tre), maltose (Mal) and *N*-acetylglucosamine (Nag) on the one hand and for sucrose (Scr) and β-glucosides (Bgl) on the other. In each, the phosphorylation product is a 6-phosphoglucopyranoside.

(a) Genes belonging to the glucose subclass. Mutations affecting the activity and the expression of the glucose PTS in *E. coli* and *S. typhimurium* have been mapped in three loci, *umgC*, *ptsG*, and *crr*. Mutations in *E. coli umgC* cause constitutive expression of $IICB^{Glc}$ and map close to its structural gene, *ptsG* (199). The gene *crr*, for the corresponding IIA^{Glc}, by contrast, maps in the *pts* operon. This separation of the structural gene for an EIICB from the gene of the corresponding EIIA is unique, since for all other PTSs the genes for all domains are clustered in a single operon. We have speculated before (358) that the reason for this separation is that IIA^{Glc} has multiple roles in the enteric bacteria. It is the essential regulatory molecule in catabolite repression and in inducer exclusion, and it is the EIIA for several other EIIs, notably those for sucrose, trehalose, and maltose (Table 2).

In *E. coli* K-12, the glucose PTS is the major glucose transport system (58). It alone is sufficient for the different glucose effects, i.e., catabolite repression, inducer exclusion, and diauxic growth caused by glucose (240). Although *ptsG* mutants grow normally on all substrates, *crr* mutants are unable to grow on a number of non-PTS substrates (see Table 3) for reasons discussed in the section on regulation by the PTS (below).

The *ptsG* gene from *E. coli* has been shown to code for $IICB^{Glc}$ by cloning the DNA and identifying the corresponding protein (29, 105). The coding sequence of *ptsG* starts with a GTG initiation codon, which is normally found in poorly translated proteins. No indication for a promoter or a repressor gene adjacent to *ptsG* has been found thus far. The *crr* genes from *E. coli* and *S. typhimurium* have also been cloned and sequenced (307, 416). In both species *crr* codes for a protein of 169 residues with 94.4% identical base pairs. This conservation may reflect the central role of IIA^{Glc} in metabolic regulation and its multiple functions.

Because *crr* maps within the *pts* operon, its gene product, IIA^{Glc}, could be considered a general PTS protein in the family *Enterobacteriaceae*. In *B. subtilis*, however, II^{Glc}, encoded by the *ptsG* gene, consists of a single protein (M_r 75,521) in which the amino-terminal part corresponds to $IICB^{Glc}$ and the carboxy-terminal part corresponds to IIA^{Glc} (140, 142, 476, 549). Unfortunately, the upstream region of the *ptsG* gene from *B. subtilis* has not yet been analyzed, and no information on its regulation is available. Immediately downstream from *ptsG* in *B. subtilis*, separated by a 101-bp intergenic sequence, are the genes *ptsH* and *ptsI*. These are expressed from a constitutive promoter, *ptsHp*. At present, additional transcription from a promoter preceding *ptsG* cannot be excluded.

Trehalose has been recognized as a PTS substrate in enteric bacteria and in *Vibrio* species (26, 228, 275, 357) and requires IIA^{Glc} for transport and phosphorylation (27). *E. coli* grown at high osmolarity synthesizes internal trehalose as an osmoprotectant through a gluconeogenic pathway (*ots* genes [139]). Under these conditions, the intracellular inducer trehalose 6-phosphate is rapidly degraded by a hydrolase. As a consequence, no induction of the *tre* genes is possible (211).

Maltose seems to be a PTS-carbohydrate in some gram-positive bacteria but is a non-PTS substrate in enteric bacteria. Recent data (372) indicate that, when overexpressed, the *E. coli malX* gene, which is apparently controlled by a repressor (*malI* gene), allows *ptsG ptsM* double mutants to grow on glucose and allows maltose transport-

negative strains to grow on maltose. These results and the sequence data showed that *malX* codes for a protein which closely resembles $IICB^{Glc}$ (35% identical residues). Its natural substrate and function have yet to be identified.

Nag and its analogs (including the antibiotic streptozo[to]cin) are taken up through EII^{Nag} (200, 240, 528). Its structural gene, *nagE*, is part of the *nag* regulon (Fig. 4), which has been sequenced completely from *E. coli* (336, 338, 342, 345, 399) and to a large extent from *K. pneumoniae* (511).

(b) Genes belonging to the sucrose subclass. Genes involved in sucrose utilization through a PTS-dependent pathway have been studied extensively in three gram-positive organisms (*B. subtilis*, *Streptococcus mutans*, and *L. lactis*) and in two gram-negative organisms (*K. pneumoniae* and *Vibrio alginolyticus*). Furthermore, a sucrose regulon is found on large conjugative plasmids (e.g., pUR400) from *E. coli* and *Salmonella* strains. In each system, sucrose is taken up through a sucrose PTS to yield intracellular sucrose 6-phosphate, which is cleaved by an intracellular invertase to yield glucose 6-phosphate and fructose.

The *scr* regulon, located on pUR400, consists of two operons, *scrK* and *scrYAB* (130, 158, 438). One operon contains the gene *scrK*, encoding an ATP-dependent fructokinase (13), and a second operon contains the genes *scrY*, encoding a sucrose- and glucan-specific porin (159, 437), *scrA*, encoding EII^{Scr} (89), and *scrB*, encoding an invertase which hydrolyzes sucrose 6-phosphate with a high affinity and sucrose with a low affinity. The reading frames of *scrA* and *scrB* overlap in a way (TAATG) that indicates translational coupling. The *scr* regulon from pUR400 is closely related to one found on a large conjugative Sac plasmid (for saccharose utilization) from *Salmonella thompson* (53) and to a *scr* regulon on the chromosome of *K. pneumoniae* (463, 519). These three *scr* regulons also resemble the *scr* system of *V. alginolyticus* (24).

Sucrose metabolism has been well studied in certain streptococci, considered the principal causative agents of dental caries. During growth on sucrose, they synthesize, by means of glucosyltransferases, water-insoluble glucans essential in bacterial colonization of tooth surfaces. Sucrose transport occurs via a II^{Scr} (encoded by *scrA*) and hydrolysis of sucrose phosphate by an invertase (encoded by *scrB*). These genes are linked but are divergently transcribed (433).

In *L. lactis*, the genes *scrA* (encoding II^{Scr}), *scrB* (encoding an invertase), and *scrK* (encoding a D-fructokinase I) reside on large conjugative transposons (Tn*5306* and Tn*5307*) (486, 487). The three genes are closely linked (gene order *scrKAB*). The gene order and enzyme properties reveal many features common to the *scr* systems of gram-negative bacteria.

Perhaps the most complex sucrose system is found in *B. subtilis* (for reviews, see references 64, 215, and 466). Unfortunately, the placement of genes from at least two metabolic pathways (*sac* and *lev*) and of a pleiotropic regulatory system (*deg*) into one presumed *sac* "regulon" has caused confusion. Sucrose metabolism first involves an extracellular β-D-fructofuranosyl transferase or levansucrase (encoded by *sacB*) (122), which, in its precursor form, contains a signal peptide. Its synthesis is regulated by an antitermination mechanism which involves an antiterminator (encoded by *sacY*) and a regulatory EII^{Sac} (encoded by *sacX*) (551). Also involved is an EII^{Scr} transport system (460 amino acids, encoded by the *sacP* gene), which lacks a IIA domain and resembles the II^{Scr} from gram-negative bacteria (about 50% identical residues) (120, 121), and an intracellular sucrase, which is closely related to all invertases characterized thus far.

Many strains of enteric bacteria cannot utilize β-glucosides because the corresponding genes are cryptic, i.e., intact but not expressed in wild-type cells (153, 435). However, mutations arise spontaneously that restore growth on β-glucosides. Four different systems have been identified, three of which involve a PTS, in *E. coli*, *K. pneumoniae*, and *Erwinia carotovora* (225, 226, 320). Most mutations which activate the cryptic *E. coli bgl* operon (*bglGFB* [Fig. 4]) are IS*1* and IS*5* insertions upstream of the *bgl* promoter (391, 392, 439, 442). Inactivity of the operon in wild-type strains is due to the weakness of the *bglGp* promoter (formerly *bglR*); low activity is caused or strongly reinforced by local supercoiling of the chromosomal DNA. Thus, the IS insertions act as mobile enhancers by altering this supercoiling. The first gene of the operon, *bglG*, encodes an antiterminator protein which is modulated by the activity of II^{Bgl}, the product of the second gene, *bglF*. Since the mechanism of antitermination is found in several PTS operons, it will be discussed in detail below. A very similar β-glucoside utilization system (*arb*) is found in *Erwinia chrysanthemi*, a phytopathogenic bacterium, but in this case the operon is not cryptic (93).

(ii) Mannitol class. (a) Genes belonging to the mannitol subclass. The genes involved in mannitol catabolism in *E. coli* are clustered in the *mtlADR* operon (459). The operon codes for an EII^{Mtl} (*mtlA*) and a mannitol 1-phosphate dehydrogenase (*mtlD*), both apparently regulated by a repressor (*mtlR*) (237, 247). The genes *mtlD* and *mtlA*, but not *mtlR*, have been sequenced (61, 196, 235). II^{Mtl} (637 residues) is a large EII of the class IICBA, whose topology and mechanism have been analyzed in great detail as discussed above.

S. aureus, *S. carnosus*, and *S. mutans* contain a soluble IIA^{Mtl} (M_r ca. 15,000) which resembles the last 145 residues from IIA^{Mtl} of *E. coli* (117, 175, 370). Its equivalent gene (*mtlF*) in *Enterococcus faecalis* (145 residues, 41% identical amino acids) maps in a cluster (probably an operon or regulon), together with *mtlD* for a mannitol 1-phosphate dehydrogenase, *mtlA* for a $IICB^{Mtl}$ (505 residues), and *orfX* of unknown function (the order is *mtlA orfX mtlFD*) (119). The gene *mtlA*, which encodes $IICB^{Mtl}$ of *S. carnosus* (505 residues), codes for a close relative of the $IICB^{Mtl}$ domains of *E. coli* (43% identical residues) (118). The $IICB^{Mtl}$ and IIA^{Mtl} proteins of gram-positive organisms and their genes, *mtlA* and *mtlF*, respectively, which always map together with *mtlD*, thus clearly resemble the *mtl* operon of the enteric bacteria and its encoded proteins.

In enteric bacteria, all three naturally occurring hexitols, mannitol, glucitol (or sorbitol), and galactitol (or dulcitol), are PTS carbohydrates (237). The similarity in their metabolic pathways and in the apparent gene order within the corresponding operons (Fig. 4) had originally prompted speculations about a common ancestor (238, 247). Although sequence data for the *gatADR* operon are still not available, the *gut* operon revealed interesting and puzzling differences from the *mtl* operon (544, 545). The *E. coli gutABDMR* operon consists of the structural genes *gutA* [$II(CB)^{Gut}$], *gutB* (IIA^{Gut}), and *gutD* (glucitol 6-phosphate dehydrogenase; 259 residues). Although $II(CB)^{Gut}$ (506 residues) plus IIA^{Gut} (123 residues) clearly are the equivalent of the enteric II^{Mtl}, no similarity can be found at the DNA or amino acid level [the designation $II(CB)^{Gut}$ is used to indicate that the order of the domains cannot be determined at present].

(b) Genes belonging to the fructose subclass. D-Fructose can be taken up in enteric bacteria by more than one PTS.

Only one of these, however, is linked at the genetic level with the corresponding catabolic genes. This PTS generates fructose 1-phosphate during transport and is induced by fructose. Only this system will be called a fructose PTS. Mannose is also a low-affinity substrate of this system (K_m > 5 mM). The accumulated phosphate ester is mannose 6-phosphate, however (222). According to sequence alignment studies, the closest relative of the fructose PTS is the mannitol PTS. No intergenic complementation with members of the glucose class has been found, and only local similarities between proteins of the classes can be found.

In enteric bacteria analyzed thus far, the *fru* operon (*fruFKA*) consists of three genes, encoding (i) a protein FPr (*fruF*), (ii) a fructose 1-phosphate kinase (*fruK*), and (iii) an EII^{Fru} (*fruA*) (134, 316, 367). The *fru* operon from *R. capsulatus* contains the genes *fruBKA*, *fruB* (coding for MTP as discussed above), *fruK* (encoding a fructokinase), and *fruA* (encoding II^{Fru}) (541, 542). The *fruA* and *fruK* genes have also been cloned from *X. campestris* and sequenced (68, 69). The enteric *fru* operon is regulated by a repressor (composed of 334 amino acids) (134, 194, 221, 358) which binds, as inducers, fructose 1-phosphate with a high affinity and fructose with a low affinity (193). FruR-negative mutations cause the constitutive expression of the *fru* operon and cause sensitivity toward xylitol and L-sorbose, two analogs taken up through the fructose PTS. They complement *ptsH* mutants to a Pts^+ phenotype and prevent growth on lactate and pyruvate. The complex role of FruR in gluconeogenesis and catabolite repression, which explains these observations, is discussed in the section on the fructose PTS and PEP synthase (below).

(iii) Lactose class. In most gram-positive bacteria and in a few plasmid-containing strains of enteric bacteria, galactose and the disaccharide lactose are PTS carbohydrates. These form a family with the cellobiose PTS.

(a) Genes belonging to the lactose subclass. The lactose and galactose pathways are important for gram-positive lactic acid bacteria, in which the corresponding genes are often found on large metabolic plasmids, since these bacteria are specialized for the fermentation of lactose in dairy products (43, 282). PTS-dependent metabolism of lactose and galactose in gram-positive bacteria yields free glucose and/or galactose 6-phosphate. This latter compound is isomerized to tagatose 6-phosphate and is metabolized via a tagatose 6-phosphate kinase to tagatose 1,6-bisphosphate and subsequently via an aldolase to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (409, 502). Interestingly, part of this pathway (phosphofructokinase B, encoded by *pfkB*, and ketose-bisphosphate aldolase, encoded by *kba*), is found in strains of enteric bacteria. It is also involved in the degradation of the PTS substrate galactitol through tagatose 6-phosphate (239, 276).

(b) Genes belonging to the cellobiose subclass. *E. coli* K-12 contains a cryptic *celABCDF* operon for cellobiose degradation, which can be activated by spontaneous mutations (321, 322). *celD* encodes a repressor inducible by cellobiose, arbutin, or salicin, whereas *celF* encodes a phospho β-glucosidase. Interestingly, CelD and CelF are similar to the melibiose-specific repressor and α-galactosidase from *E. coli*. The genes *celABC* encode the domains IIB^{Cel}, IIC^{Cel}, and IIA^{Cel}, respectively.

(iv) Mannose class. Three highly similar PTSs are the mannose (Man) and L-sorbose (Sor) PTSs from enteric bacteria and a fructose (Lev) PTS from *B. subtilis*. They contain two soluble and two membrane-bound domains or proteins, comprising about 850 amino acids. All three accept fructose as a major substrate.

The mannose PTS has an unusually broad substrate specificity. In enteric bacteria it is the only effective transport system for mannose, hence its name. It also transports *N*-acetylglucosamine, glucosamine, 2-deoxyglucose, glucose, and fructose. Although the mannose PTS in *S. typhimurium* is as efficient as the glucose PTS in uptake and phosphorylation of glucose, in *E. coli* K-12 it is a minor system for this substrate (58, 198, 240, 471). Genetic analyses, as well as cloning and sequencing data, have indicated the presence in *E. coli* of a single locus for the mannose PTS made up of three genes (*manXYZ*, formerly *ptsLMN*) encoding three polypeptides, which have been discussed previously (104, 107, 429–431, 529). The *man* operon is controlled by the NagC repressor (345, 510).

In *B. subtilis*, a levanase able to hydrolyze sucrose and fructose polymers is encoded by *sacC* (correctly, *levC*). It is part of a *lev* operon, inducible by fructose, together with four additional ORFs (*levDEFG*) encoding a fructose PTS (278). The *lev* operon is preceded by an ORF, LevR (938 residues), whose complex structure and possible role in regulation are discussed below.

Finally, the ketose L-sorbose is metabolized in many enteric bacteria via a PTS (462, 538). The genes from the chromosome of *K. pneumoniae* and of a naturally occurring strain of *E. coli* have been cloned and have been sequenced completely for *K. pneumoniae* (520, 535; submitted to the EMBL data base by U. F. Wehmeier as accession number X66059) and partially for *E. coli*. Uptake through the L-sorbose PTS (genes *sorFBAM*) yields L-sorbose 1-phosphate, which is degraded through a reductase (*sorE*) to glucitol 6-phosphate, and through a dehydrogenase (*sorD*) to fructose 6-phosphate (208, 461).

Regulation of Synthesis of PTS Proteins

As expected for a system as central for bacterial metabolism as the PTS, synthesis of both its general and its specific proteins is regulated in a highly sophisticated way. Generally, the PTS operons and regulons are inducible by the major substrate (Table 2) and in addition are subject to global regulatory control.

Mechanisms regulating expression of the *pts* operon. In enteric bacteria, expression of the *pts* operon increases about threefold during growth on PTS substrates and requires an intact cyclic AMP (cAMP)-cAMP receptor protein (CRP) system (72, 280, 390, 471). Anaerobic growth conditions also favor a higher expression (246), in agreement with a hypothesis that the PTS is the major carbohydrate transport system under anaerobiosis (406). Many strains that contain polar or deletion mutations in the *ptsH* and *ptsI* genes express the *crr* gene at normal levels (52, 445, 446). This complex regulation, which involves several promoters and different regulatory proteins, has been analyzed recently in some detail. Three, possibly four, promoters may be involved in the expression of the *pts* operon of *E. coli*. Two of these promoters (P0 and P1) are located upstream of *ptsH*, separated by about 100 bp. A third one (P2) is located within the 3′-terminal end of *ptsI*. It is the major promoter of *crr* (74, 125). A fourth transcription start site, possibly involved in antitermination, is located downstream of *ptsH* and is directed against P0 and P1 (252). According to in vitro binding tests, P0 and P1 include a high-affinity and a low-affinity CRP-binding site, respectively. DNase I footprinting experiments revealed that P1 is the major promoter in the

absence of the cAMP-CRP complex and that P0 is the major promoter in its presence (74, 125). These results were extended to in vivo conditions. The P0 and P1 promoters (in *cis*) are controlled by CRP, since fusions of the chloramphenicol acetyltransferase gene to these promoters show increased chloramphenicol acetyltransferase activity (125). A second study also used fusions between the *pts* promoters and the *lacZ* gene cloned on low-copy-number plasmids as indicators, as well as P0 and P1 mutations and RNA primer extension studies (72, 74). It was shown that one major transcript encompassed the entire *pts* operon and that a shorter one encompassed only *ptsH* in the presence of the cAMP-CRP complex at the P0 site. In the absence of CRP, P0 is occluded. In the presence of CRP, binding of CRP to the P0 site is increased by P1. The results also showed that P2 is the major promoter of *crr* and is not activated by the cAMP-CRP complex. The short *ptsH* mRNA and the short *crr*-specific mRNA account for 80% of the total *ptsH* and *crr* mRNA, respectively, thus explaining the relative abundance of HPr and IIA^{Glc} in a cell compared with EI, as was observed earlier by quantitating the proteins (280, 517). The data also explain why IIA^{Glc} is synthesized at wild-type levels in polar and deletion *ptsHI* mutants. Whether transcription termination, the *ptsH* antisense RNA, or processing is involved in the production of these short mRNAs is unknown.

Transcription of the *pts* operon is enhanced by unphosphorylated $IICB^{Glc}$ produced during glucose transport through this PTS (72, 73, 75). This stimulation was also seen after overproduction of II^{Glc} but not after accumulation of intracellular glucose (from melibiose or maltose) or glucose 6-phosphate. The target of the II^{Glc}-mediated stimulation is the CRP-binding site of P0 itself or an overlapping sequence. The glucose- and the CRP-mediated regulations seem to be alternative ones, never acting simultaneously in a cell. They are poised to keep *pts* expression at a similar level under conditions of low and high catabolite repression. On the basis of a weak sequence similarity between $IICB^{Glc}$ and the transmitter module of two-component systems (217), a two-component system which would involve glucose as the external signal, II^{Glc} as the sensor, and an as yet unidentified component as the second regulator for *pts* expression has been proposed (73). The only other *pts* operon studied is that of *B. subtilis* (142). The *ptsH* and *ptsI* genes constitute an operon, and, similar to *E. coli*, the *ptsH* gene is also transcribed in the opposite sense.

Mechanisms regulating expression of inducible PTS operons. One would expect the operons for PTS-dependent catabolic pathways to be induced either from the outside by free substrate in the medium or by intracellular intermediates, e.g., the carbohydrate phosphates generated through transport and phosphorylation. A precedent for the former mechanism is the induction of the *uhp* uptake system for hexose phosphates by external glucose 6-phosphate (182) and other two-component systems. These act via the transfer of a histidine-bound phosphate from a membrane-bound sensor protein kinase to its regulator protein. This allows the latter to activate transcription from specific promoters (470).

As discussed in detail in our previous review (358), there have been many indications for induction from inside by intracellular PTS carbohydrate phosphates and hydrolysis products but, unexpectedly, also for induction from inside by free PTS carbohydrates. Recent molecular data obtained through sequencing the corresponding promoters/operators and regulatory proteins and through identifying the molecular inducers begin to reveal the mechanisms involved, as discussed in the next section.

(i) Regulation through classical regulator-operator pairs. The majority of inducible catabolic genes analyzed thus far in eubacteria are regulated in a negative way by repressor-operator pairs or in a positive way through activator-initiator pairs. In rare cases, a regulatory protein has both repressor and activator functions.

Sequencing of the *nag* genes from *E. coli* and from *K. pneumoniae* revealed two divergently transcribed operons (336, 342, 510); both are transcribed from a central CRP-binding site and two opposing promoters, as shown by gel retardation assays and by footprinting (342, 343, 345). The first operon expresses *nagE* for the II^{Nag} at a semiconstitutive level, whereas the second contains, in addition to the structural genes for catabolic enzymes (Fig. 4), a gene called *nagR* in *K. pneumoniae* (510) and *nagC* in *E. coli* (342). This gene encodes a repressor (344, 345, 510, 512). The *nag* repressor binds *N*-acetylglucosamine 6-phosphate (and perhaps glucosamine 6-phosphate) as the inducer (343) and recognizes operators that are fully conserved between *E. coli* and *K. pneumoniae* (511). In agreement with these results, it had been observed previously that mutants which lack *N*-acetylglucosamine 6-phosphate deacetylase and hence cannot degrade *N*-acetylglucosamine 6-phosphate become fully constitutive (240, 528) and that an intact II^{Nag} or II^{Man} is required for induction from outside (240, 343).

Interestingly, the *E. coli nag* repressor also controls the mannose PTS (510) through an operator which closely resembles the operators of the *nag* regulon (345). Similar to the *N*-acetylglucosamine PTS, it acts apparently as a scavenger system for amino sugars generated during cell wall synthesis and turnover and thus should be expressed continuously. Glucosamine 6-phosphate deaminase, involved in further amino sugar metabolism, must be strictly regulated, however, to avoid a futile cycle involving the amino sugar biosynthetic enzymes (glucosamine 6-phosphate synthase [344, 512]). *nag* regulation is also dependent on the cAMP-CRP complex. The DNA of the *nag* regulatory region has an intrinsic curvature which is enhanced by binding of two NagC repressor molecules to both promoters simultaneously. This curvature is converted into a loop on binding of the cAMP-CRP complex (345). The bent DNA is not found in front of the *manXYZ* operon. For the *man* operon, another regulatory factor is required for expression under anaerobic conditions (398). The *dgsA* gene has been cloned (299) but has not yet been analyzed in any detail.

The *scr* regulons from enteric bacteria are all regulated by a repressor, ScrR, whose structural gene, *scrR*, is transcribed independently from the other *scr* genes (193, 436). On the basis of gel retardation assays, free intracellular fructose and, to a lesser degree, fructose 1-phosphate were identified as molecular inducers. Neither sucrose 6-phosphate (or sucrose) nor glucose 6-phosphate could act as inducers. It had been observed previously (438) that wild-type cells could be induced by sucrose and fructose-containing oligomers, provided an intact sucrose 6-phosphate hydrolase was present. Hydrolase-negative mutants were inducible only by fructose (438). Expression of *scrK* was completely independent of the cAMP-CRP complex, whereas expression of the *scrYAB* operon was fully dependent on CRP (436, 438).

ScrR is a member of the GalR-LacI repressor family (521) with a characteristic helix-turn-helix motif. A characteristic operator motif is present once in the *scrK* promoter and twice, together with a CRP-binding site, in the promoter of

the *scrYAB* operon (13, 53, 159). The presence of low-activity promoters for *scrA*, *scrB*, and *scrR*, each one located in the preceding structural gene, and a strict translational coupling between *scrA* (encoding $IIBC^{Scr}$) and *scrB* (encoding hydrolase) indicates an even more complex regulation.

E. coli and *S. typhimurium* FruR is a close relative of ScrR (about 42% identical residues [194]). It binds fructose 1-phosphate, the immediate product of the fructose PTS, with high affinity and free fructose with a low affinity (193). This might explain previous data which indicated the presence of endogenous induction of the *fru* operon in *E. coli* mutants lacking fructose 1-phosphate kinase (113, 127).

The three hexitol-specific systems of enteric bacteria are regulated by repressors (70, 237, 247, 459). The *E. coli gut* operon contains two (*gutMR*) and possibly three (*gutQ*) regulatory genes, *gutR* encoding a repressor and *gutM* encoding an activator (546). This confirms previous data which were based on a genetic analysis (57, 247, 281). GutR and GutM seem to act antagonistically and to compete for a common operator. On induction, GutR is inactivated and thus increases the synthesis of the activator GutM from the main promoter, a process which is dependent on the cAMP-CRP complex. The model proposed explains at the same time the delayed induction (ca. 25 min) of the *gut* operon (247) and the preferred utilization of mannitol when cells are grown in the presence of both mannitol and glucitol (236). Tight *ptsI* mutants of *E. coli* can still be induced from outside as well as from inside by mannitol (supplied in the form of galactosyl-mannitol through LacY and hydrolysis by β-galactosidase [460]) and by glucitol (247) for the *mtl* and the *gut* operon, respectively. This seems to indicate that intracellular free hexitol can act as an inducer. Other data indicate that in *E. coli*, mannitol 1-phosphate produced either through mannitol 1-phosphate dehydrogenase from fructose 6-phosphate (482) or through an as yet unidentified pathway (408) causes a permanent endogenous induction of the *mtl* operon. The physiological function would be the regulation of NAD(H) pools during growth on reduced-carbon sources. Possibly, the substrate-phosphate acts as a high-affinity inducer and the free substrate acts as a low-affinity inducer.

A final example of regulation by classical regulator-operator pairs is the *sor* operon of *E. coli* and *K. pneumoniae*, in which a single regulatory protein, SorC, maps within the operon (520, 536). In the absence of sorbose, SorC acts as a repressor ($SorC_R$) and is converted during induction into the activator configuration ($SorC_A$). This model is based on the analysis of *sorC* mutations which lock the SorC molecule in the repressor or the activator configuration. The sequence of SorC (EMBL data base accession no. X66059) shows that it is a member of the so-called LysR-regulator family (167). The *sor* operon also shows delayed induction (up to 20 min [462]) reminiscent of the delayed induction of the *gut* operon, where activator (GutM) and repressor (GutR) are also found but in two different proteins as described above.

On the basis of sequence analyses and/or on analyses of mutants with an altered regulation, repressors have also been invoked for other PTS operons. These include the *lac* system of *L. lactis* (501) and *S. aureus* (317, 318), with galactose 6-phosphate (300) or a derivative as the putative inducer, and the *cel* operon of *E. coli*, with *celD* (the gene encoding the repressor) within the operon (Fig. 4) (321).

(ii) Regulation through antiterminator proteins. In a number of cases, the expression of PTS operons is regulated by transcription antitermination. We will discuss this type of regulation in some detail for the *bgl* operon of *E. coli* and the *sac* and *lev* operons of *B. subtilis*. Induction from outside has been described for these systems. In each case, the corresponding EII acts as the membrane-bound sensor. The IIA and IIB domains can phosphorylate the intracellular regulator molecules (e.g., BglG in Fig. 5). After phosphorylation through EI, HPr, IIA, and IIB, these regulator molecules, e.g., P-BglG, are inactive. During uptake of a PTS substrate, antiterminators are dephosphorylated through backward flow of the phospho groups to IIB and IIA and the substrate, and they thus prevent termination of transcription. Mutations which prevent the PTS-dependent phosphorylation of the antiterminator, e.g., those inactivating EI, HPr, IIB, or IIA, thus cause a characteristic constitutive expression of operons regulated via this mechanism (64, 132, 364).

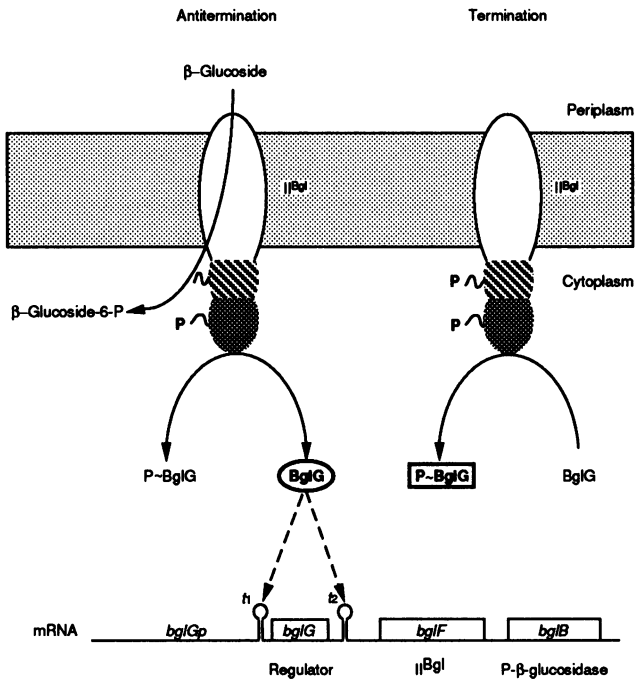

FIG. 5. Schematic model of antitermination in the *bgl* operon. The *bglGp* promoter preceding the genes *bglG* (antiterminator BglG), *bglF* (II^{Bgl}) and *bglB* (phospho β-glucosidase) is constitutive. Transcription is terminated partially by terminators t1 and t2, thus allowing a residual synthesis of BglG and II^{Bgl}. In the absence of an inducer, II^{Bgl} phosphorylates and, as a consequence, inactivates BglG completely: the operon is repressed. In the presence of a substrate of the β-glucoside PTS, phospho-BglG is dephosphorylated at the expense of substrate phosphate, and the activated BglG prevents termination and thus causes induction of the *bgl* operon.

As summarized in Fig. 5, the *bgl* operon is expressed in decryptified *E. coli* strains from a constitutive promoter (272, 442). The first gene, *bglG*, is flanked on both sides by terminators t1 and t2. BglG (278 residues) can be phosphorylated in a reversible reaction by II^{Bgl} (7, 439). The phosphorylated amino acid in BglG and the immediate phospho donor (IIB or IIA?) are still unknown. BglG is an inactive monomer in its phosphorylated form (8). During uptake of β-glucosides, the phospho group is transferred preferentially from P-II^{Bgl} to its substrate. This drain causes the dephosphorylation of P-BglG and the subsequent dimerization of BglG to an active antiterminator. BglG binds to specific RNA sequences located upstream of t1 and t2 (176), prevents termination, and thus causes induction of the *bgl* operon (272, 442). The model is supported by mutations in

ptsI, *ptsH*, and *bglF* (II^{Bgl}), which all cause a constitutive expression of the *bgl* operon, and by mutations in *bglG* which prevent transcription. BglG may also be phosphorylated at the expense of IIA^{Glc}. This cross-regulation between II^{Glc}, II^{Bgl}, and BglG, coupled to a CRP-dependent control of the *bgl* promoter, causes strong catabolite repression of this operon in the presence of glucose (440).

Elements of an antitermination mechanism for catabolic operons were first described in the complex *sac* regulon of *B. subtilis* (450, 466; for reviews, see references 64 and 215). The first *sac* operon encoding levansucrase (*sacB*) is expressed from a constitutive promoter, but transcription stops at a terminator (previously called *sacR*) immediately upstream of *sacB*. The *sacY* gene encoding the corresponding antiterminator is localized in a separate operon together with the *sacX* gene encoding $IIBC^{Scr}$, which has only a regulatory role. Pts^- mutants express *sacB* constitutively. An antitermination mechanism, involving SacX as a sensor and SacY as the antiterminator, has been proposed by analogy to the *bgl* system (56, 63, 551) (Fig. 5). A second *sac* operon comprises a gene encoding a sucrase (*sacA*) and one encoding $IIBC^{Scr}$ (*sacP*), which is active in transport, and apparently is also regulated through an antiterminator, SacT, encoded by the *sacT* gene. The sensor (SacP?) has yet to be identified (63). The antiterminators SacT and SacY are similar to each other and to BglG. The $EIIBC^{Scr}$s (SacP and SacX) resemble each other (63, 465). Despite these similarities, other data suggest that SacT requires a functional PTS and possibly an intracellular inducer for its activity (11). The role of this inducer would be to prevent phosphorylation of SacT at a second phosphorylation site and thus to prevent its function as an antiterminator. Transcription of the *sacXY* operon is inducible by sucrose and is regulated by the DegS-DegU (formerly *sacU*) two-component regulatory system (55).

A third system of *B. subtilis* affected by mutations in the *pts* operon is the Lev system for degradation of levans and other fructose polymers (278). Because a levanase encoded by *levC* (formerly *sacC*) also hydrolyzes sucrose, it had been classified previously as a member of the *sac* regulon. The *lev* operon, which also contains the genes *levDEFG* encoding the fructose PTS, is controlled by a regulator, LevR (encoded by *levR*). It behaves as a repressor in the uninduced state and as an activator after induction with fructose (64). Compared with other regulators with a dual role, e.g., SorC (315 residues) or FruR (334 residues), LevR (938 residues) is a very large protein. Two domains have been identified in LevR. One domain (residues 144 to 345) shows extensive similarity to NifA/NtrC regulators. These regulators are required for the formation of an open promoter complex by σ^{54} and the RNA-polymerase holoenzyme. The NifA/NtrC regulators also contain an ATP-binding site. An upstream activating sequence (UAS) and the so-called $-12/-24$ consensus sequence form the promoter of the *lev* operon. All these elements are found in LevR and in the *lev* promoter. Furthermore, mutants lacking σ^{54} (encoded by *sigL*) no longer express the *lev* genes (65, 66). A second domain of LevR (residues 411 to 572) resembles the antiterminators SacT, SacY, and BglG. The presence of this domain is surprising because no transcriptional terminator could be found in the *lev* promoter. Instead, it was found that phosphorylation of LevR through EI/HPr and the soluble components IIA^{Lev} and IIB^{Lev} prevents its normal configuration as an activator. *levG* mutants with a defect in IIC^{Lev} can no longer be induced. Phosphorylation-defective mutants (e.g., with mutations in *ptsHI* or in *levDE*), on the other hand, show a constitutive expression of the *lev* operon. Furthermore, uptake of fructose through the PTS causes dephosphorylation and induction (64).

REGULATION BY PTS

As mentioned above, mutants defective in EI and/or HPr are unable to utilize PTS carbohydrates as the sole source of carbon for growth. However, it was also observed that such *ptsHI* mutants were unable to grow on a number of non-PTS carbon sources. In the enteric bacteria these carbon sources include lactose, maltose, melibiose, glycerol, Krebs cycle intermediates, rhamnose, and xylose. Although this phenomenon is less well characterized in other bacteria, it is known, for instance, that *B. subtilis ptsI* mutants do not grow on maltose and glycerol, which are non-PTS carbohydrates in this organism as in enteric bacteria. Quite some effort has been invested in understanding and explaining how the absence of the general proteins of the PTS or the presence of a PTS carbohydrate affects growth on a range of non-PTS carbon sources. This section will deal with these phenomena, which include catabolite repression, regulation of cAMP synthesis, and regulation of solute transport.

As discussed in our previous review, when bacteria are exposed to different carbon sources, one is frequently used preferentially and growth occurs in two phases (diauxie; for detailed references, see reference 358). The preferred substrate prevents the uptake and metabolism of the less preferred substrate by decreasing the amount and/or the activity of the corresponding uptake system and/or the first metabolic enzyme. Although glucose repression is often cited as the paradigm, many compounds can prevent the uptake and metabolism of others, provided that the rate of their catabolism surpasses the rate of their anabolism. Any growth condition which causes an excess of catabolism over anabolism can cause catabolite repression. Catabolite or permanent repression is partial (at most 50 to 80% repression) but lasts as long as the repressing compound is present. Transient repression, on the other hand, which occurs during conditions of shift-up or diauxic growth, is more severe (80 to 100% repression) but lasts only from 0.1 to 1 doubling time. Although exogenous cAMP can overcome catabolite repression completely and transient repression partially, there is no direct relationship between the level of this compound and the degree of repression; i.e., other processes must play a role. One of these processes is inducer exclusion, the inhibition of the activity of an uptake system by a preferred substrate or one of its metabolic derivatives. We will discuss in this section the role of the PTS in these phenomena.

Several observations are crucial in understanding the phenotype of *ptsHI* mutants of members of the *Enterobacteriaceae* with respect to non-PTS carbon sources. Pastan and Perlman (330) reported that growth of an *E. coli ptsI* or *ptsH* mutant on the non-PTS carbohydrate lactose could be restored by addition of cAMP in the growth medium, similar to stimulation of growth of a *cya* mutant, lacking adenylate cyclase (Table 3). Furthermore, it was found that mutations in repressor genes and promoter-up mutations restored the growth of *E. coli pts* mutants on single non-PTS carbon sources such as glycerol (for references, see reference 358). *S. typhimurium crr* (carbohydrate repression resistant) mutations were isolated that restored growth of *ptsI* mutants on several non-PTS carbon sources simultaneously (Table 3) (422, 423). Since most studies have concentrated on *E. coli* and *S. typhimurium*, we will first discuss the molecular basis

TABLE 3. Phenotype of *ptsI*, *ptsH*, and *crr* mutants of enteric bacteria

Genotype	Additions	Growth on[a]:		
		PTS carbohydrates[b]	Class I compounds[c]	Class II compounds[c]
Wild type	–	+	+	+
ptsH	–	–[d,e]	–	–
ptsI	–	–[c]	–	–
ptsHI	–	–[c]	–	–
ptsI (leaky)	–	–	+	+
ptsI (leaky)	PTS carbohydrate	–	–	–
ptsHI crr	–	–	+	–
*ptsHI crp**	–	–	+	+
ptsHI	cAMP	–	+	+
crr	–	+	+	–
*crr crp**	–	+	+	+
crr	cAMP	+	+	+
cya	–	–[f]	–	–
cya	cAMP	+	+	+

[a] Growth is scored on minimal medium plates containing 0.2% of the carbon source. +, growth after 48 h at 37°C; –, no growth.

[b] PTS carbohydrates include glucose, fructose, mannose, hexitols, β-glucosides, trehalose, *N*-acetylglucosamine, glucosamine, sorbose, sucrose, and cellobiose.

[c] Class I compounds include lactose, melibiose, maltose, and glycerol; class II compounds include Krebs cycle intermediates, xylose, rhamnose, and galactose via the methyl β-galactoside permease.

[d] *ptsH* mutants grow on fructose.

[e] Of the three best-studied species, *E. coli*, *S. typhimurium*, and *K. pneumoniae*, *ptsHI* mutants of only *K. pneumoniae* can grow on glucose because of the presence of an alternative glucose-oxidizing pathway consisting of glucose dehydrogenase and pyrroloquinoline quinone (303). The occurrence of this pathway in the different species has been studied (31).

[f] *cya* mutants grow slowly on glucose and fructose.

of these phenomena in enteric bacteria. Then we will discuss whether the mechanisms responsible for these phenomena in enteric bacteria are more widespread and can be used to explain similar behavior in other bacteria.

To simplify the following discussion of PTS-mediated regulation, we will begin by presenting a model for PTS-mediated regulation in enteric bacteria. In Fig. 6 the essential elements of PTS-mediated regulation are shown. The central regulatory molecule is IIA^{Glc} (formerly called III^{Glc}), which can exist in two states: phosphorylated IIA^{Glc} ($P\text{-}IIA^{Glc}$) and nonphosphorylated IIA^{Glc}. The phosphorylation state of IIA^{Glc} is determined by the balance between phosphorylation via P-HPr and dephosphorylation via $IICB^{Glc}$ in the presence of its substrate. IIA^{Glc} and $P\text{-}IIA^{Glc}$ interact with and regulate different proteins. IIA^{Glc} binds to and inhibits several enzymes essential in carbohydrate metabolism, e.g., the lactose and melibiose carriers, the MalK component of the maltose transport system, and glycerol kinase. The direct result is inhibition of uptake and of subsequent metabolism of these carbon sources. This process is called inducer exclusion. The phosphorylation state of IIA^{Glc} is involved in the activation of adenylate cyclase. Since cAMP is required for the expression of many catabolic genes, regulation of the cAMP level allows control of enzyme synthesis. Inducer exclusion and regulation of cAMP synthesis can enhance each other. In a cell growing on a non-PTS carbohydrate, the PTS proteins most probably will be in the phosphorylated forms. Addition of a PTS carbohydrate will increase the flux of phospho groups and lower the phosphorylation states of the PTS proteins, including IIA^{Glc}. Under those conditions, the entry of the non-PTS carbon source is inhibited (high concentration of IIA^{Glc}) and expression of the catabolic genes is prevented (low concentration of $P\text{-}IIA^{Glc}$).

In the next sections we will discuss the data supporting such a model and its implications for cellular metabolism. Phenotypes of *pts* and *crr* mutants and the effect of PTS carbohydrates on growth are summarized in Table 3 and also will be explained in detail in the next sections. Two classes of carbon sources will be considered. Class I includes lactose, melibiose, maltose, and glycerol; class II includes Krebs cycle intermediates, xylose, and rhamnose.

Inducer Exclusion

Transport and metabolism of class I non-PTS carbohydrates, i.e., lactose, melibiose, maltose, and glycerol, is inhibited by PTS carbohydrates (Table 3). Although inhibition can be observed in certain wild-type $ptsH^+$ $ptsI^+$ strains, the effect is much stronger in leaky *ptsI* strains containing only residual EI activity. Experiments performed with these strains and nonmetabolizable substrates of the lactose and melibiose permease, such as thiomethylgalactoside (TMG), showed that in these cases transport per se is inhibited (422). Inhibition can be brought about by any PTS carbohydrate, as long as its corresponding EII is present at a sufficiently high level. The model depicted in Fig. 6 offers an explanation. Any PTS carbohydrate can dephosphorylate $P\text{-}IIA^{Glc}$, either directly via $EIICB^{Glc}$ for glucose or indirectly because transport and phosphorylation of all other substrates via their respective EII complexes will result in dephosphorylation of P-HPr. Since the phosphorylation of IIA^{Glc} by P-HPr is a reversible process, $P\text{-}IIA^{Glc}$ will be dephosphorylated as a consequence (see the section on mechanisms of PTS-mediated transport and phosphorylation).

The role of IIA^{Glc} in inducer exclusion is well established. Binding of purified IIA^{Glc} to the various target proteins has been shown in a number of cases. IIA^{Glc} binds to the lactose carrier (309, 319). In liposomes reconstituted with the purified lactose permease, TMG transport was inhibited by the addition of IIA^{Glc} (309). In membrane vesicles made from an *S. typhimurium* mutant that contained no EI, HPr, or IIA^{Glc} and expressed the lactose carrier from a plasmid, transport of TMG was inhibited when vesicles were reconstituted with purified IIA^{Glc} (292). These experiments also showed a difference between the two forms of IIA^{Glc}, IIA^{Glc}_{Slow} and IIA^{Glc}_{Fast} (286) (see the section on phosphotransfer mechanisms of EIIs, above). Whereas IIA^{Glc}_{Slow} inhibited TMG uptake, IIA^{Glc}_{Fast}, lacking the first seven amino-terminal amino acids, had a smaller effect (292). Binding of IIA^{Glc} to glycerol kinase and inhibition of its activity have been shown (67, 314, 356). In the maltose transport system, binding of IIA^{Glc} to the MalK component has been reported (355), in support of the genetic data (417). In proteoliposomes made from membranes that overexpressed the maltose transport system, inclusion of IIA^{Glc} in the liposomes inhibited maltose transport to a maximum of 60% (62). Although binding of IIA^{Glc} to the melibiose permease has not been shown yet, this interaction will most probably resemble that of the lactose permease. It was shown in a vesicle system that inclusion of IIA^{Glc} in vesicles inhibited the melibiose carrier (292).

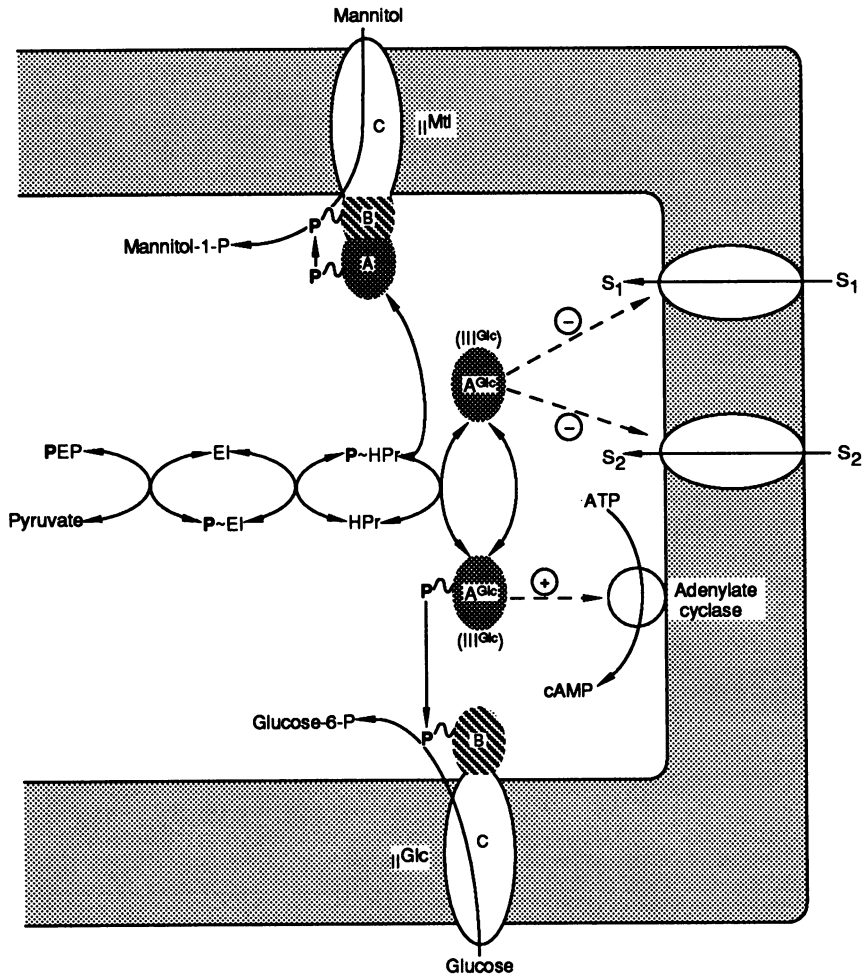

FIG. 6. Model for regulation by the PTS. In addition to the general proteins of the PTS, two EIIs are shown, those specific for mannitol (II^{Mtl}) and for glucose (II^{Glc}). Activation (+) of adenylate cyclase by phosphorylated IIA^{Glc} and inhibition (−) of two different non-PTS uptake systems, S1 and S2 (e.g., for lactose, melibiose, maltose, or glycerol), by nonphosphorylated IIA^{Glc} are also indicated. Other symbols as in Fig. 1.

Two features of IIA^{Glc}-mediated inducer exclusion are similar, regardless of the target protein. (i) Nonphosphorylated IIA^{Glc} binds to the target proteins, and phosphorylation of IIA^{Glc} prevents its binding. (ii) Binding of IIA^{Glc} occurs only when a substrate of the target protein is present, e.g., a β-galactoside, glycerol, or maltose (see below). For the MalK protein, the complete maltose transport system, including the maltose-binding protein, must be present for maltose-induced binding of IIA^{Glc} to MalK. These observations suggest that a conformational change is required before IIA^{Glc} can bind.

The *crr* mutation first was isolated because it restored the growth of *ptsHI* mutants on all class I non-PTS carbohydrates simultaneously (Table 3). Elimination of the general inhibitor, IIA^{Glc}, can explain this phenotype. It is important to note that the *crr* mutation (in contrast to the *crp** mutation or addition of cAMP) restores growth of *ptsHI* mutants only on class I compounds but not on class II compounds (Table 3). This is in agreement with the observation that class II systems are not sensitive to inducer exclusion.

Another type of mutant had been isolated previously in which transport and metabolism of a single, specific class I carbon source was not inhibited in the presence of a PTS carbohydrate. These mutations mapped in or closely linked to the gene(s) encoding the corresponding catabolic pathway (427). Most probably they represent altered target proteins to which IIA^{Glc} cannot bind any longer, although in only one case has this been shown in binding studies with purified IIA^{Glc} (355). A number of these mutations in the *lacY* (355, 530), *malK* (62, 229), and *melB* (233) genes have been sequenced recently and may help in establishing which residues or domains are recognized by IIA^{Glc}. At present, no definitive conclusion can be drawn. Although there is some similarity between short stretches of amino acids in these target proteins, in which mutations are located that eliminate inducer exclusion, a number of mutations have been found far outside these regions. A case in point is the MalK protein. Mutations that eliminate inducer exclusion have been found in regions covering about two-thirds of the protein (62, 229).

Still other specific suppressor mutations have been found. As mentioned above, promoter-up mutations have been found, e.g., mutations in the *glp* operon that allow *E. coli ptsI* mutants to grow on glycerol (18). Another type of suppressor mutation resulted in a glycerol kinase that had

become insensitive to feedback inhibition by fructose 1,6-bisphosphate (17).

Different *crr* mutant alleles have been isolated. In a number of cases these mutations lead to the absence or lowered synthesis of IIA^{Glc} (287, 446). These types of mutations reveal very little about the nature of interaction between IIA^{Glc} and its target proteins. In a few studies, mutations have been isolated in the *crr* gene that resulted in IIA^{Glc} molecules that were defective in regulation but still active in glucose transport and phosphorylation. In addition, mutations have been introduced by site-directed mutagenesis. A replacement of the active-site His residue (His-75) by glutamine resulted not only in a IIA^{Glc} that could not be phosphorylated but also in a protein that was defective in regulation (365). Zeng et al. (550) recently isolated *E. coli crr* mutants that were resistant to inducer exclusion but still had a functioning IIA^{Glc}. Three different substitutions were found: G47S, A76T, and S78F. The mutation in the *iex* allele (324), previously shown to result in a temperature-sensitive IIA^{Glc} which is defective in binding to the lactose carrier at the nonpermissive temperature (304), is due to the G47C replacement (84a).

An exciting recent result is the determination of the three-dimensional structure of the complex of *E. coli* IIA^{Glc} with glycerol kinase (181). From the observation that neither the binding of chloroplatinate anion to the active-site His (540) nor the phosphorylation of IIA^{Glc} (332) resulted in large structural changes, it was concluded that phosphorylation of the active-site His residue (His-90), i.e., the introduction of negative charge, is sufficient to render IIA^{Glc} inactive in interacting with proteins of class I uptake systems. The three-dimensional structure of the IIA^{Glc}-glycerol kinase complex shows that the tetrameric glycerol kinase binds four IIA^{Glc} molecules. The contact between both molecules is limited to a rather small area, involving residues 402 and 472 to 481 of glycerol kinase and 38 to 46, 71, 78, 88, 90, and 94 to 97 of IIA^{Glc}. The contact consists of a very hydrophobic environment in which residues 472 to 481 of glycerol kinase project directly into the active site of IIA^{Glc}. Phosphorylation of His-90 would lead to direct electrostatic and steric repulsion between the covalently bound phospho group and glycerol kinase. This would disrupt the binding between IIA^{Glc} and glycerol kinase. The IIA^{Glc}-binding site is far removed from the active site of glycerol kinase. Some predictions have been made about residues in glycerol kinase involved in binding to IIA^{Glc} (62). These residues, 370 to 385, are quite different from those identified from the crystal structure as being involved in binding. The residues identified in IIA^{Glc} as possibly involved in interaction with non-PTS proteins (550) are included in the residues mentioned above, however.

Regulation of Adenylate Cyclase Activity

cAMP plays a central role in gene expression in enteric bacteria. Together with CRP, it is involved in the (generally) positive regulation of a number of catabolic genes. Mutants defective in either adenylate cyclase (*cya* mutants) or CRP (*crp* mutants) are unable to grow on a range of carbon sources (for a recent review, see reference 28). Addition of external cAMP restores growth of *cya* but not of *crp* mutants on these carbon sources. It has been known for a long time that the overproduction of cAMP is deleterious to *E. coli* cells. Consequently, the level of cAMP is strictly controlled, both at the level of transcription and translation of the *cya* gene and at the level of activity of adenylate cyclase.

A more direct link between cAMP synthesis and the PTS, suggested by the stimulation of growth of both *cya* and *ptsHI* mutants of *E. coli* on certain non-PTS compounds by cAMP (330), was provided by the observation of Peterkofsky and coworkers (160, 339) that adenylate cyclase activity in toluenized *E. coli* cells is strongly inhibited by PTS carbohydrates. This inhibition is dependent on the presence of the EII specific for a particular carbohydrate. On the basis of the behavior of *ptsHI* mutants and the assumption that the presence of a PTS carbohydrate lowers the phosphorylation state of the PTS enzymes, it was suggested that the phosphorylated form of EI, P-EI, acts as an activator of adenylate cyclase. Later experiments showed that the *crr* gene product, $P\text{-}IIA^{Glc}$ (or IIA^{Glc}) is involved rather than EI, since in *crr* mutants containing normal amounts of EI, adenylate cyclase activity is low (114, 306). It is important to point out that *crr* mutants and *ptsHI* deletion mutants have a residual level of cAMP synthesis. This contrasts with *cya* deletion strains, which are defective in cAMP synthesis. Unfortunately, a demonstration of the direct stimulation in vitro of adenylate cyclase by $P\text{-}IIA^{Glc}$ (with or without additional EI and HPr) has not been successful (368). In fact, addition of EI, HPr, and IIA^{Glc} to bacterial extracts inhibited adenylate cyclase activity. Addition of only EI also inhibited this activity, but addition of HPr stimulated it. Phosphorylation of all PTS proteins by adding PEP had no effect unless phosphate, an activator of adenylate cyclase, was present. Addition of αMG lowered the adenylate cyclase activity at most twofold when the PTS proteins and PEP were present (368). Strangely, the adenylate cyclase activity was higher in extracts containing only PEP than in extracts containing the PTS proteins and PEP, i.e., the phosphorylated form of the proteins. In a later paper (255) it was shown that, in the presence of phosphate, nonphosphorylated IIA^{Glc} inhibited adenylate cyclase. It had been previously suggested, on the basis of studies with mutants, that IIA^{Glc} might be an inhibitor of adenylate cyclase in addition to $P\text{-}IIA^{Glc}$ being an activator (306, 360).

Could most of the regulation of adenylate cyclase activity be at the level of expression? In our previous review (358) we cited experiments which showed that the expression of *cya* is inhibited by the cAMP-CRP complex. However, various groups reported different levels of inhibition, ranging from less than 2-fold to more than 10-fold. Recently, a similar study was conducted with the *cya* gene from *S. typhimurium* (111). It was shown conclusively, by using *cya-lac* fusion strains, that one of the *cya* promoters, P2, is negatively regulated by the cAMP-CRP complex and that the decrease in *cya* transcription under repressing conditions is about fourfold. These and previous results point to the fact that adenylate cyclase is regulated at the level of transcription but that this effect is not sufficient to explain the large changes observed in cAMP synthesis under various conditions. It should be realized that the rates of cAMP synthesis and secretion into the medium can vary over at least a 100-fold range (201). Moreover, PTS carbohydrates can inhibit cAMP synthesis in toluenized cells by 10- to 20-fold. Therefore, adenylate cyclase activity and cAMP synthesis have to be regulated much more than the twofold range observed in the in vitro system. There are several possible explanations for the failure to reconstitute the regulation of adenylate cyclase in vitro. It might be difficult to mimic the intracellular conditions, although adenylate cyclase was overproduced by using a cloned *cya* gene and the PTS proteins were added at concentrations approaching intracellular concentrations. Another unknown (protein) factor

could be involved. Although elongation factor Tu (369), GTP (464), and the membrane electrochemical potential (340) each affect adenylate cyclase activity, these effects are too small to explain the large variations in cAMP synthesis observed under various conditions. In this context it should be mentioned that Gershanovitch et al. (137) have presented some data suggesting that FPr, the *fruF* product, is involved in cAMP synthesis. Adenylate cyclase activity in *E. coli fruF* mutants was 20 to 25% of the wild-type activity (in contrast, Feldheim et al. [112] reported a stimulation of adenylate cyclase activity in *fruB*::Mu mutants lacking FPr). Introduction of a *fruF*::Mu dJ insertion into a *ptsH* mutant also lowered cAMP production 2.5-fold in this double mutant (112). Although it was claimed that the *ptsH fruF* double mutant produced extremely low levels of cAMP, the actual production was 20% that of the wild-type strain, i.e., similar to that of a tight *ptsI* or *crr* mutant.

Mutants that lack CRP produce large amounts of cAMP (362). This increased production is dependent on IIA^{Glc}, since it is absent in *crr* mutants (54, 71). Mutants have been isolated with a modified regulation of cAMP synthesis (54). Several mutations in the *E. coli cya* gene that abolish the stimulation of cAMP synthesis in *crp* strains have been described. Possibly, interaction between IIA^{Glc} and adenylate cyclase is altered. Interestingly, introduction of the *Pasteurella multocida cya* gene in an *E. coli crp* strain also leads to an increased level of cAMP synthesis compared with the *crp crr* double mutant. Perhaps a similar IIA^{Glc}-adenylate cyclase interaction is present in *P. multocida* (297).

It can be concluded that the proposed role for $P\text{-}IIA^{Glc}$ as an activator of adenylate cyclase is based mainly on genetic arguments. In our opinion, there is at present no biochemical proof that IIA^{Glc} interacts directly with adenylate cyclase, either alone or in a complex with EI and HPr, as suggested by Peterkofsky et al. (for a review, see reference 341). Nor is there any evidence that adenylate cyclase is phosphorylated by $P\text{-}IIA^{Glc}$ or any other PTS protein. However, there is no doubt from the results discussed above that IIA^{Glc} is somehow involved in the regulation of adenylate cyclase.

Details of PTS-Mediated Regulation

In the previous section we discussed how the absence of the general PTS proteins EI and HPr or the presence of nonmetabolizable PTS analogs can affect the growth of *E. coli* or *S. typhimurium* on class I carbon sources. The defects could be relieved by the addition of cAMP (or, alternatively, by introduction of a *crp** mutation) or by the introduction of a *crr* mutation. How can changes in two such different processes, i.e., cAMP synthesis and inducer exclusion, both restore growth on these class I compounds?

For inducer exclusion to occur, a number of factors are important: (i) the number of IIA^{Glc} molecules; (ii) the number of target protein molecules; (iii) the phosphorylation state of IIA^{Glc} (and other PTS proteins); (iv) the presence of a substrate of the target protein; and (v) the intracellular cAMP level, which is partly determined by the phosphorylation state of the PTS proteins but also determines the level of expression of CRP-cAMP-dependent genes. These factors are discussed below.

(i) and (ii) Since IIA^{Glc} and its target protein form a stoichiometric complex, the number of protein molecules and the dissociation constants are important. An *E. coli* or *S. typhimurium* cell contains 1×10^4 to 2×10^4 IIA^{Glc} molecules (25 to 50 μM [446]). Considering that the K_D of the lactose permease-IIA^{Glc} complex is between 5 and 16 μM (309), most if not all lactose permease molecules can be complexed, resulting in almost complete inhibition of lactose transport. The number of lactose carriers in an induced *E. coli* cell is estimated to be 8×10^3 per cell (0.2 nmol/mg of membrane protein). The number of melibiose carriers is of the same order of magnitude. Thus, an *E. coli* cell should contain sufficient IIA^{Glc} to complex all lactose carriers. Similarly, from the amount of glycerol kinase (approximately 15 μM [164]) and the K_i of IIA^{Glc} (10 and 4 μM at pH 7 and 6.5, respectively [314], and somewhat higher at pH 7.5 [67]), it can be calculated that sufficient IIA^{Glc} is available to inhibit most glycerol kinase molecules. In the maltose transport system, estimates of the number of MalK proteins range between 10^3 and 10^4 molecules per cell (452). No kinetic details are known, as in the case of the melibiose transport system.

Are these calculations borne out by the available experimental data? It has been shown that both increasing the amount of IIA^{Glc} above the chromosomal level (295, 305) and decreasing the amount of a target protein by partial induction (306, 419; see also reference 49) render cells more sensitive to inducer exclusion. Similarly, decreasing the amount of IIA^{Glc} or increasing the number of target molecules, for instance by inducing more than one class I system (305), has the opposite effect: cells escape from inducer exclusion. These results support the model shown in Fig. 6. Still, it remains very difficult to predict whether, under a particular set of conditions, cells will be sensitive to inducer exclusion. A simple example is the sensitivity of wild-type $ptsH^+$ $ptsI^+$ cells. In most cases, the glucose analogs 2DG, αMG, and thioglucose inhibit growth of *E. coli* cells on class I compounds whereas the routinely used *S. typhimurium* strains are not sensitive unless they contain a leaky *ptsI* mutation. The same conclusion can be drawn from uptake experiments. Growing an *S. typhimurium* strain on maltose or glycerol, i.e., inducing the respective catabolic operons fully, results in cells in which glycerol or maltose uptake is not inhibited by 2DG or αMG (by reducing the level of glycerol kinase or the maltose transport system, inducer exclusion returns, however [306]). These observations do not fit well with predictions based on the numbers discussed above. Thus, other factors, discussed below, must also be important in establishing inducer exclusion.

(iii) All data point to an essential role of nonphosphorylated IIA^{Glc} in inducer exclusion. The phosphorylation state of IIA^{Glc} is determined by the rate of phosphorylation via P-HPr and the rate of dephosphorylation via $IICB^{Glc}$ (for glucose) and HPr (for all other PTS carbohydrates). Using the different mobilities of IIA^{Glc} and $P\text{-}IIA^{Glc}$ in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, it was shown that in a wild-type *S. typhimurium* strain, addition of αMG resulted in dephosphorylation of $P\text{-}IIA^{Glc}$ from roughly 80% to less than 10% (308). No other data are available about the phosphorylation state of IIA^{Glc} in intact cells.

Another approach toward analyzing which factors determine the percentage phosphorylation of the various PTS proteins was recently initiated by using metabolic control analysis (163, 203). In essence, the control of each enzyme on a metabolic pathway, the control coefficient, C_E^J can be determined by changing the activity of that enzyme, E_i, while keeping all others constant, and by measuring the change in flux, J, through the pathway in terms of the change in enzyme activity, E_i ($C_E^J = [dJ/J]/[dE/E]$). If the change in flux divided by the change in enzyme activity ($C_E^J = [dJ/J]/[dE/E]$) is 1, the enzyme has total control. If $C_E^J = 0$,

the enzyme has no control at all; i.e., increasing its activity has no effect on the flux. In the case of a linear pathway, the sum of all control coefficients C_E^J of the enzymes involved is 1. Control coefficients of IIA^{Glc} and $IICB^{Glc}$ on glucose uptake have been determined recently (359, 412). Whereas IIA^{Glc} has no control ($C_E^J = 0$), $IICB^{Glc}$ has a control coefficient of 0.65. It has been reported that a large overproduction of EI (20- to 70-fold) does not affect the uptake of αMG or 2DG in *E. coli* (138), but in these experiments it has not been checked whether the amounts of all other PTS enzymes remained constant. Although the C_E^J values of HPr and EI have not yet been determined, these results suggest that at least one important factor in determining the phosphorylation state of IIA^{Glc} is the rate at which the phospho group can be transferred from P-IIA^{Glc} to $IICB^{Glc}$. It is thus possible that dephosphorylation of P-IIA^{Glc} via $IICB^{Glc}$ is a "slow" step, resulting in high steady-state levels of P-IIA^{Glc} in the absence or presence of PTS substrates. Therefore, other factors, e.g., EI, HPr, or PEP, must be important, since this is contrary to the notion that glucose is effective in inducer exclusion because it causes the dephosphorylation of P-IIA^{Glc} to IIA^{Glc}.

(iv) The observation that IIA^{Glc} binds only to its target protein when a substrate is present can be rationalized by concluding that IIA^{Glc}, being present at a constant and limited level in the cell, is not being wasted on nonproductive binding. It has been shown that induction of a second target protein, e.g., glycerol kinase, into a cell already containing a certain level of the maltose transport system results in less inhibition of the maltose system by 2DG only if glycerol is present, i.e., if glycerol kinase can bind IIA^{Glc} and can lower the free concentration of IIA^{Glc} (305).

(v) Although adenylate cyclase activity is certainly regulated by the phosphorylation state of the PTS proteins, in particular IIA^{Glc}, the actual cAMP concentrations in the cell and in the medium, as well as the consequences on the expression of different genes under various conditions, are difficult to predict. First, different genes and operons require different concentrations of cAMP for full expression (219, 258, 479). This may explain why *crr* mutants (which still contain a low but significant residual adenylate cyclase activity) can grow on certain carbon sources (class I) but not on others such as succinate, citrate, or xylose (class II [Table 3]). Expression of genes required for class I carbon source catabolism in general requires less cAMP than does expression of those of class II carbon sources. However, the range over which changes in the intracellular cAMP concentration have significant effects is rather narrow. Experiments by Epstein et al. (98) showed that lowering the intracellular cAMP concentration fivefold, from 2.5 to 0.5 μM, lowers the differential rate of β-galactosidase synthesis from 30 to less than 10 U/mg. The effects of these relatively small changes may explain the different phenotypes of *ptsHI-crr* deletion mutants of *E. coli* and *S. typhimurium* that are sometimes observed. As explained above, the *crr* mutation was originally isolated in *S. typhimurium* as a suppressor of the PtsHI-negative phenotype, i.e. restoration of growth on maltose, glycerol, and melibiose (423). Similar mutations with a similar phenotype were also isolated in *E. coli* (41, 304). Recently, a set of isogenic *E. coli* mutants containing *ptsI-crr* and *ptsHI-crr* deletions were constructed (253). Those mutants were unable to grow on class I compounds, in contrast to the *S. typhimurium* and *E. coli* mutants described above. Growth of the *E. coli ptsHI-crr* mutant on class I compounds was stimulated by cAMP. In these particular *E. coli* strains, the cAMP level was only 3% of that of the parent whereas the adenylate cyclase activity in *S. typhimurium crr* and *ptsHI-crr* strains and in some other *E. coli* mutants may be 10 to 20% of that of the parent (114, 306). Thus, the background of a particular strain might be important and may explain the observed differences.

From the above, it is clear that at present it is almost impossible to predict how a particular strain or mutant will respond when supplied with a mixture of carbon sources, especially mixtures of PTS and non-PTS carbohydrates. Depending on the level of transport systems already present and the internal cAMP concentration, catabolism of the non-PTS carbohydrates might be rapid or might be inhibited completely by PTS carbohydrates.

IIA^{Glc}-Like Molecules

Most evidence points to a central role of IIA^{Glc} in the regulation of carbohydrate metabolism in enteric bacteria. Mutations in other EIIs do not lead to the pleiotropic effects associated with the *crr* mutation. The recent cloning and sequencing of a number of genes encoding EIIs from various organisms revealed that some contain carboxy-terminal domains resembling IIA^{Glc} (see the section on structures of the PTS proteins, above). In the enteric bacteria this applies to EII^{Nag} and EII^{Bgl}. However, similar IIA^{Glc}-like domains are also found in gram-positive organisms: in II^{Glc} of *B. subtilis* (142) and in II^{Scr} of *S. mutans* (433), each of which has a carboxy-terminal domain resembling IIA^{Glc}. Finally, a similar IIA^{Glc}-like domain is found in a non-PTS transport system, the lactose carrier, LacS, of *Streptococcus thermophilus* and *Streptococcus bulgaricus* (348), which is an active transport system involving proton symport. LacS contains two His residues (His-537 and His-552), which are conserved in all IIA^{Glc} domains. Phosphorylation of wild-type LacS, but not of a mutant LacS lacking His-552, has been demonstrated. Lactose transport, however, was not affected by mutations in these His residues (347). An obvious question is whether these IIA^{Glc}-like domains can function like the normal enteric IIA^{Glc} molecule, either in glucose transport and phosphorylation or in regulation.

By using *crr* mutants lacking αMG transport, it was shown that both II^{Nag} and II^{Bgl} (the latter when decryptified) could restore αMG transport, i.e., replace IIA^{Glc} (507). Conversely, IIA^{Glc} can restore *N*-acetylglucosamine transport in a mutant in which the carboxy terminus, comprising the IIA^{Glc}-like domain, is truncated (509). These experiments show that interaction of two EIIs is possible, i.e., the transfer of a phospho group from IIA^{Glc} to $IICB^{Nag}$ or from IIA^{Nag} to $IICB^{Glc}$. In analogous experiments it was reported that the carboxy-terminal domain of II^{Bgl} could transfer its phospho group to $IICB^{Glc}$ and that IIA^{Glc} could complement a mutant II^{Bgl} in which the active-site His was replaced by an Arg or Asp residue (441).

The existence of another IIA^{Glc}-like domain may also explain the observation that *S. typhimurium crr* mutants can still transport αMG at 10 to 20% of the rate of the parent strain (354, 355). Elimination of II^{Nag} did not affect αMG uptake in such mutants, but mutations linked to the locus that contains the *cel* operon in *E. coli* were found that abolished the residual αMG transport (354, 355). The sequence of the *E. coli* EII^{Cel} contains no IIA^{Glc}-like domain, however (321).

The gene fragment encoding the IIA^{Glc} domain of *B. subtilis* EII^{Glc} was cloned and shown to complement growth on glucose of an *E. coli crr* mutant that also lacked the mannose PTS (141). This IIA^{Glc} domain also restored αMG

transport in the same *E. coli crr* mutant (140). Introduction of this domain into an *E. coli ptsHI-crr* deletion strain resulted in inhibition of fermentation, whereas the synthesis of β-galactosidase in an *E. coli crr* strain containing the IIA^{Glc} domain became sensitive to glucose repression (388). Thus, the *B. subtilis* equivalent of the enteric IIA^{Glc} restores PTS-mediated regulation in a *crr* mutant.

A soluble fraction of *Clostridium acetobutylicum* can complement αMG phosphorylation in an extract of an *E. coli crr* mutant, suggesting that this organism also contains a IIA^{Glc}-like molecule (296). It cannot be excluded, however, that *C. acetobutylicum* contains an EII^{Glc} like *B. subtilis*, in which the IIA^{Glc}-like domain has been partially cleaved off by endogenous proteolytic activity.

Except for the functional complementation of *crr* mutants by the *B. subtilis* IIA^{Glc} domain, complementation of the regulatory function of IIA^{Glc} by other EIIs containing a IIA^{Glc} domain has not yet been shown. *crr* mutants containing active II^{Nag} or II^{Bgl} do not regain growth on succinate, suggesting that the phosphorylated IIA^{Glc}-like domains cannot sufficiently activate adenylate cyclase (507). Inducer exclusion was not found in the *E. coli crr* mutants containing II^{Nag} or II^{Bgl}, although an *S. typhimurium crr* mutant, lacking all IIA^{Glc}, still showed inducer exclusion of glycerol and maltose by 2DG (306).

Catabolite Repression in Nonenteric Bacteria

The involvement of IIA^{Glc} in PTS-mediated regulation of metabolism in members of the *Enterobacteriaceae* is well established. One might ask whether the PTS, if present, also has a regulatory function in other bacteria and, if so, whether IIA^{Glc} plays a similar role. As mentioned above, *B. subtilis ptsHI* mutants show pleiotropic effects, comparable to those of enteric bacteria. A *B. subtilis ptsI* mutant does not grow on maltose and glycerol, and αMG (glucose) inhibits the uptake of glycerol or maltose in a mutant containing a thermosensitive EI which has been inactivated by heating (378, 480). A double mutant containing both a *ptsHI* deletion and a truncated *ptsG* gene, resulting in an $EIICB^{Glc}$ that lacks the IIA^{Glc}-like domain, was constructed. This mutant does not regain growth on glycerol or maltose (140), however, which makes it unlikely that IIA^{Glc} in *B. subtilis* functions as it does in enteric bacteria. It should be pointed out that *B. subtilis* synthesizes cAMP only under special (oxygen-limited) conditions (271). This observation makes a role of cAMP in catabolite repression in *B. subtilis* unlikely. Therefore, the phenotype of *B. subtilis ptsI* mutants may resemble that of similar *E. coli* mutants but the mechanism underlying this regulation may be quite different. It has been reported that a *ptsI* mutant of *S. aureus* is unable to grow on the non-PTS carbohydrate maltose (38). However, this system has not been studied further.

One possible mechanism of PTS-mediated repression in gram-positive bacteria may involve phosphorylation of non-PTS target proteins. It was reported that dihydroxyacetone (glycerol) kinase of *Enterococcus faecalis* can be phosphorylated by P-HPr on a His residue (76, 83). The phosphorylated enzyme is activated approximately 10-fold. Phospho dihydroxyacetone kinase can also transfer its phospho group to HPr (83). This would offer another way in which to regulate the uptake of non-PTS carbohydrates. Indeed, it has been reported that glucose and its analog 2DG inhibit glycerol uptake in *E. faecalis*, in particular in leaky *ptsI* mutants (401). One obvious explanation is that lowering the level of P-HPr reduces the level of phosphorylation of dihydroxyacetone kinase because EII complexes and dihydroxyacetone kinase compete for P-(His)-HPr. It should be remembered, however, that the same phenotype in enteric bacteria is caused by inhibition of glycerol kinase by nonphosphorylated IIA^{Glc}. Phosphorylation of glycerol kinase from *Bacillus stearothermophilus* has also been reported (380). It is not known whether the glycerol-negative phenotype of *B. subtilis ptsI* mutants (see above) can be explained in a similar way.

Our knowledge about the mechanisms involved in catabolite repression in nonenteric bacteria is very limited. In a number of organisms, *cis* elements that are involved in catabolite repression, both upstream of the start codon and in the coding sequences, have been detected (123, 168, 234, 311, 415, 457, 522). In *S. aureus* the *lac* genes, encoding the lactose PTS, are repressed by glucose. Regulation is at the level of transcription, and both the LacR repressor and the unidentified catabolite repressor are thought to bind to the *lac* promoter sequence (318). Except for a *B. subtilis* transacting protein that is involved in catabolite repression of α-amylase and shows similarity to the LacI and GalR repressors of *E. coli* (168), no other gene products have been identified that are responsible for the regulation of various catabolite-repressible genes in nonenteric bacteria. Moreover, no evidence exists for a role of IIA^{Glc}-like domains in regulation in the other bacteria containing this molecule.

Conclusions

The classic observations by Monod (298) on diauxic growth of *E. coli*, i.e., the preferential use of one carbon source over another, can be explained in a qualitative sense to a large extent by the mechanisms we have discussed in this section. Many PTS carbohydrates are utilized in preference to other carbon sources, since they induce a net dephosphorylation of IIA^{Glc}. This leads in turn to a lower expression of the catabolic operons (less cAMP as a result of less P-IIA^{Glc}) and (in some cases) inhibition of non-PTS uptake systems already synthesized. It is important to point out that not only in *ptsHI* and *crr* mutants but also in wild-type strains growing on PTS carbohydrates, adenylate cyclase activity and cAMP synthesis are not decreased to zero (in contrast to the situation in *cya* mutants). This residual cAMP level allows enteric bacteria to grow on certain carbon sources but not on others. Most importantly, this basal cAMP level allows wild-type cells to grow on PTS carbohydrates, many of which require cAMP. Otherwise, growth on a PTS carbohydrate would be self-inhibitory. Preference among PTS carbohydrates is possible via competition of the various EIIs for P-HPr (85, 86, 444).

As with nonenteric bacteria, catabolite repression in enteric bacteria must involve more than the PTS. Epstein et al. (98) demonstrated a correlation between the intracellular cAMP level and the level of β-galactosidase activity. Growth on some carbon sources, such as glucose 6-phosphate and gluconate, results in relatively low cAMP levels, but there is no satisfactory mechanism to explain this phenomenon. In *E. coli* strains lacking EI, both glucose 6-phosphate and glycerol can lower the intracellular cAMP concentration and thus catabolite and transient repression can result (548). Obviously, adenylate cyclase activity and/or the steady-state cAMP level can be regulated in the absence of changes in the PTS proteins.

OTHER INTERACTIONS BETWEEN PHOSPHOTRANSFERASE AND NONPHOSPHOTRANSFERASE SYSTEMS

In the previous section we described the interaction between IIA^{Glc}, several non-PTS uptake systems, and adenylate cyclase, resulting in inducer exclusion and regulation of cAMP synthesis. In the last few years a number of other processes have been described in which proteins of the PTS are involved in the regulation of various metabolic reactions or are phosphorylated by other non-PTS proteins. We will discuss in this section (i) inducer expulsion in gram-positive organisms; (ii) regulation of gluconeogenesis and other processes by the repressor of the fructose PTS; (iii) interaction between EI, acetate kinase, and possibly other kinases; (iv) a possible link of the PTS with nitrogen regulation; and (v) poly(β-hydroxybutyric acid) accumulation in *Alcaligenes eutrophus*. Chemotaxis toward PTS carbohydrates, which also must involve interaction of the PTS with a number of non-PTS proteins, will be discussed in the next section.

Inducer Expulsion in Gram-Positive Organisms

As discussed above, HPr of gram-positive organisms can be phosphorylated on two different residues. A His residue can be phosphorylated by P-EI, and a Ser residue can be phosphorylated by an ATP-dependent HPr kinase. Whereas the phospho group of P-(His)-HPr can be transferred to the various EIIs, the phospho group of P-(Ser)-HPr is removed by a phosphoprotein phosphatase and has been implicated in the regulation of carbohydrate transport.

PTS carbohydrates such as glucose inhibit the uptake of TMG via the lactose PTS in many lactic acid bacteria (for a review, see reference 484). In *L. lactis* this inhibition has been attributed to the preferential utilization of P-(His)-HPr by the mannose PTS during glucose transport and phosphorylation (489). It was noted in subsequent studies that addition of glucose or a few other PTS carbohydrates to *Streptococcus pyogenes* cells that had previously accumulated phosphorylated β-galactosides resulted in the intracellular dephosphorylation of the β-galactoside phosphates and the appearance of the free galactoside in the medium (379). This process, called inducer expulsion, has subsequently been demonstrated for a number of gram-positive organisms. It requires energy in the form of ATP (377). In conjunction with the observation that, in an extract of *S. pyogenes*, HPr can be phosphorylated on a Ser residue by an ATP-dependent protein kinase (82), it was suggested that P-(Ser)-HPr might be involved in the regulation of carbohydrate uptake and efflux in gram-positive organisms. Phosphorylation of a Ser residue in HPr of various gram-positive organisms has been demonstrated. The site of phosphorylation in *Enterococcus faecalis* HPr was determined to be Ser-46 (81).

A possible role of P-(Ser)-HPr in regulation was suggested by various lines of evidence. (i) Phosphorylation of P-(Ser)-HPr by P-EI is much slower than that of nonphosphorylated HPr (79). In the presence of PEP and EI, the ATP- and protein kinase-dependent phosphorylation of HPr on the Ser residue is also inhibited (78). Formation of doubly phosphorylated HPr is thus difficult. Nonetheless, P-(His)-P-(Ser)-HPr has been demonstrated in extracts of oral streptococci (493).

(ii) The very slow phosphorylation in vitro of *L. lactis* P-(Ser)-HPr by PEP plus EI can be enhanced by the addition of (heterologous) EIIAs such as IIA^{Glu} (from *E. faecalis*) or IIA^{Lac} (from *S. aureus*) (79). Thus, in an intact cell, phosphorylation of the Ser residue does not necessarily prevent or inhibit the flux of phospho groups via histidine, i.e., transport and phosphorylation via the PTS. In a later study with proteins from *B. subtilis*, phosphotransfer from PEP plus EI to HPr occurred at a higher rate in the presence of IIA^{Glc} than when IIA^{Mtl} was present (388). Although the rate of phosphotransfer from PEP plus EI to a mutated S46D HPr was about threefold lower, the ratio between rates in the presence of IIA^{Glc} and IIA^{Mtl} remained the same.

(iii) The purified protein kinase is activated by several intermediates of glycolysis, e.g., fructose 1,6-bisphosphate, glucose 6-phosphate, and 2-phosphoglycerate (82). P_i, which inhibits the protein kinase, is an activator of the *E. faecalis* phosphoprotein phosphatase which dephosphorylates P-(Ser)-HPr (80). A high level of glycolytic intermediates might thus slow down the further uptake and phosphorylation of PTS carbohydrates. This notion was supported for *S. mutans* by the observation that the uptake rates of glucose and sucrose in vivo are stimulated by starvation and correlated with the levels of P-(Ser)-HPr (260).

The possible significance of the phosphorylation of the Ser residue in HPr has been investigated by two groups using mutated proteins in which the serine was replaced by an Ala, Thr, Tyr, or Asp residue (90, 387). The experimental results are not entirely in agreement. Whereas Reizer et al. (387) found that mutant HPrs in which the serine is replaced with a threonine (S46T) or an alanine (S46A) exhibit phosphotransfer activities comparable to that of wild-type HPr, Eisermann et al. (90) reported lower activities. It was found that the growth rate on PTS carbohydrates of a mutant containing the S46A mutation was not affected although the yield of the mutant strain was about half that of the wild type (90). Similarly, it was concluded that phosphorylation of Ser-46 did not affect uptake of PTS carbohydrates, because cells containing S46A HPr (which cannot be phosphorylated) accumulated the carbohydrates at the same rate (387). In addition, inducer expulsion did not seem to be different in cells containing the S46A mutation. Inhibition of TMG uptake by various PTS carbohydrates was similar in cells containing wild-type and S46A HPr (387). From these and other experiments, the authors drew the conclusion that exclusion of one carbohydrate by another was not due to the presence of P-(Ser)-HPr but rather to competition for P-(His)-HPr. Unfortunately, all these experiments were performed with a *Staphylococcus aureus ptsH* mutant in which the mutant and wild-type HPr molecules were expressed from a plasmid containing the heterologous *B. subtilis ptsH* gene. Since the level of expression is unknown, it is difficult to predict what the level of serine phosphorylation would be in the wild-type HPr, i.e., whether sufficient nonphosphorylated HPr could be present to catalyze uptake at rates comparable to those in a wild-type strain containing chromosomal levels of HPr. Inhibition of phosphotransfer from P-EI to free HPr by P-(Ser)-HPr or S46D HPr has been excluded (388). Rather, phosphorylation of HPr on the Ser residue lowers the concentration of free HPr, thus decreasing the concentration of the species most active in PTS-mediated carbohydrate uptake.

An attempt to quantify the amounts of the different forms of HPr in streptococci has been published (493). In bacterial extracts, the various forms, HPr, P-(His)-HPr, P-(Ser)-HPr, and P-(His)-P-(Ser)-HPr, could be separated and detected by crossed immunoelectrophoresis. Whereas stationary cells grown on glucose contained mainly HPr and P-(His)-HPr, in exponentially growing cells P-(Ser)-HPr and the doubly phosphorylated HPr predominated. These results might be

expected if the hypothesis that the levels of glycolytic intermediates influence the level of P-(Ser)-HPr and thus possibly the rate of PTS-mediated carbohydrate uptake is correct (80, 82, 188, 260).

Although phosphorylation of HPr from gram-positive organisms on a Ser residue has been well established, its role in the preferential use of some carbohydrates over others is still not clear. Although certain IIA domains, e.g., IIA^{Glc}, allow faster phosphorylation of HPr by P-EI than do others, e.g., IIA^{Mtl} (388), this does not necessarily mean that glucose is the favored carbon source. The rate of phosphotransfer to the IIA domains would also be important in the overall uptake process. The slower phosphorylation of P-(Ser)-HPr by P-EI is enhanced by both IIA^{Glc} and IIA^{Mtl}, but the ratio of the rates of phosphorylation of wild-type and S46D HPr is the same (388). Thus, there is no preferential stimulation by a particular IIA domain.

How are carbohydrates expelled from the cell during inducer expulsion, and is P-(Ser)-HPr involved? Since free carbohydrate is found in the extracellular medium, the accumulated carbohydrate phosphates must be hydrolyzed intracellularly, either by reversal of the EII-catalyzed process or by phosphatases. A hexose 6-phosphate phosphohydrolase has been purified from *L. lactis* (485), and it might be involved in inducer expulsion in this organism. It is not known how the external signal, e.g., addition of glucose, regulates the phosphatase. Possibly, P-(Ser)-HPr interacts with and stimulates the phosphatase. Alternatively, the phosphatase might be regulated by covalent modification. Efflux of the free carbohydrate is the next step. For lactose and TMG efflux from *L. lactis* cells in which the phosphate ester had been accumulated, inducer expulsion was absent in mutants lacking the EII^{Lac} (386). Studies of TMG efflux in *Streptococcus pyogenes* suggested that TMG exits the cell by facilitated diffusion, catalyzed by II^{Lac} (477). TMG efflux was fast, about 10 times faster than its uptake and concomitant phosphorylation. This is contrary to the notion that facilitated diffusion of PTS carbohydrates via an EII in the absence of phosphorylation is not catalyzed rapidly (see the section on a consensus model for translocation catalyzed by the EIIs, above). However, if the proposal that the phosphorylated form of EII can catalyze the translocation of the free substrate is correct (265), efflux of free TMG via II^{Lac} would be possible since these cells contain an intact PTS. It should be pointed out that a similar process has been observed in gram-negative organisms. After αMG uptake by *E. coli* cells has reached a steady-state level, addition of tracer amounts of [^{14}C]αMG showed that the glucose analog is taken up continuously at the same rate, free αMG being expelled from the cell (150). A phosphatase has been implicated in this process (151), and efflux of the free αMG could occur via the $P\text{-}EII^{Glc}$. It is therefore possible that these processes in gram-positive and gram-negative organisms are indeed similar. Since *E. coli* lacks the protein kinase, regulation of the carbohydrate phosphate phosphatase could be different. Alternatively, P-(Ser)-HPr formation and inducer expulsion may be coincident but not causally related. It is important to point out that the correlation between protein kinase-dependent HPr phosphorylation and inducer expulsion is weak and based mostly on in vitro experiments.

Finally, it has been shown that in some heterofermentative lactobacilli, e.g., *Lactobacillus brevis* and *Lactobacillus buchneri*, HPr and an ATP-dependent protein kinase that phosphorylates HPr are present but EI and EII^{Lac} are absent (but the absence of EI was concluded from the lack of complementation of an *S. aureus ptsI* extract by an *L. brevis* extract, a heterologous system) (382). In these organisms, TMG is expelled from the cell on addition of glucose (400), as has been demonstrated for cells that contain a functional PTS. Although the process in the lactobacilli is superficially the same as that in other gram-positive organisms, it is unknown how glucose, a non-PTS substrate in *L. brevis* and *L. buchneri*, elicits TMG expulsion. It is also important to note that in these organisms, because of the absence of a complete PTS, TMG is accumulated as such and not as the phosphate ester as is the case in the other gram-positive organisms. It might be instructive to compare these phenomena with the efflux of αMG from *E. coli* on the addition of metabolizable substrates such as lactose, glycerol, or α-glycerol phosphate (for references, see reference 358).

Fructose PTS and PEP Synthase

The *fruFKA* operon of enteric bacteria encodes the enzymes involved in the fructose PTS: FPr, fructose 1-phosphate kinase, and II^{Fru}. FPr was discussed in the section on overview and PTS components (above) and can functionally replace HPr. Expression of the *fru* operon is controlled by the *fruR* gene, encoding the Fru repressor. The *fruR* gene is located at 2.2 min on the *E. coli* map, whereas the *fru* operon is localized at approximately 47 min. *fruR* mutants express the *fru* operon constitutively (134, 221, 373). *fruR* mutations can be isolated as suppressor mutations that allow *ptsH* mutants to grow on PTS carbohydrates because the constitutively synthesized FPr can complement the absence of HPr. It was noted that *S. typhimurium* and *E. coli fruR* mutants acquired an additional phenotype, the inability to grow on lactate and pyruvate (134). This was shown to be due to the complete absence, in *fruR* mutants of *S. typhimurium*, of PEP synthase, which catalyzes the conversion of pyruvate and ATP into PEP, AMP, and P_i (134). In *E. coli* approximately 30% residual activity was found but the phenotype of the *fruR* strain was similar to that of the *S. typhimurium fruR* mutant. Similar results were reported by Chin et al. (46) who also showed that PEP carboxykinase activity is lowered in *S. typhimurium fruR* mutants to about 10% of the wild-type level. It had been shown previously that a large deletion in *S. typhimurium* around the *leu* operon in the same region also resulted in the absence of PEP synthase (40). The *pps* locus, encoding PEP synthase, is located at about 37 min on the *E. coli* chromosome (35), and the *ppsA* gene has been cloned (135) and sequenced (312). Recently, another mutation, *fruS*, was described in *E. coli* which resulted in the constitutive synthesis of FPr and II^{Fru} while fructose 1-phosphate kinase was still inducible (25). The *fruS* mutation is tightly linked to *fruA* but is difficult to understand in view of the structure of the *fruFKA* operon. The *fruS* mutant, in contrast to *fruR* mutants, is still able to grow on lactate and pyruvate and contains normal levels of PEP synthase. Noncoordinate expression of *fruF* and *fruK* has also been observed in *S. typhimurium* (112). In *fruA*::Mu dJ mutants, expression of fructose 1-kinase was high but that of FPr was low.

A later study (45) showed that in *S. typhimurium*, in addition to PEP carboxykinase and PEP synthase, other enzymes involved in gluconeogenesis were affected by the *fruR* mutation. Activities of fructose 1,6-bisphosphatase, isocitrate lyase, and malate synthase in the *fruR* mutant varied from 11 to 46% of those in the parental strain. It was shown that growth in the presence of glucose had the same effect. Glycolytic enzymes such as phosphofructokinase II had an elevated activity. Although it was suggested (45) that

in *fruR* mutants glycolysis is enhanced whereas gluconeogenesis and the Krebs cycle are reduced, the pattern is not that clear. For instance, pyruvate kinase behaved like a gluconeogenic enzyme rather than a glycolytic enzyme in these experiments.

By using *fruF-galK* (133) and *ppsA-galK* (135) fusions, it was shown that transcription of both the *S. typhimurium fru* operon and the *E. coli ppsA* gene is regulated by the FruR protein. FruR acted like a repressor in the case of *fru* and as an activator for *ppsA*. Since transcription of the *ppsA-galK* fusion was not affected by a *fruF*::Tn*10* insertion mutation (135), which results in the absence of all proteins of the fructose PTS, none of the Fru proteins except FruR is required for *ppsA* activation. This makes a role of FPr as an effector of *ppsA* transcription, as suggested by Chin et al. (46), unlikely.

An unexpected effect of *E. coli* IIA^{Gut} on the activity of gluconeogenic enzymes has been reported (543). Overproduction of IIA^{Gut} by using a plasmid-encoded *gutB* gene resulted in a lowering of PEP carboxykinase (maximum sevenfold) and PEP synthase activities (twofold). At present no satisfactory model (or rationale) is available. The use of a plasmid containing the cloned *fru* operon resulted in a fourfold decrease in PEP carboxykinase activity (45). However, if FruR is an activator of genes such as *ppsA* or *pck* (encoding PEP carboxykinase), these decreases in PEP synthase and PEP carboxykinase activities are most probably due to complete titration of the activator.

The *fruR* genes of both *E. coli* and *S. typhimurium* were sequenced (194). The FruR protein contains a typical helix-turn-helix motif, common to regulatory proteins, and shows similarity to the LacI, GalR, PurR, RbtR, and DeoR repressors. Thus the same effector protein regulates transcription of the *fru* operon negatively and that of the *ppsA* gene positively.

Interestingly, PEP synthase is a phosphohistidine enzyme (50, 302), as are the PTS enzymes. Sequence analysis of the *E. coli ppsA* gene (312) showed that PEP synthase shows similarity to both EI and to pyruvate, orthophosphate dikinases (PPDK) from plants and microorganisms (346). The active-site region, identified in PPDK and EI, is also conserved in PEP synthase. PEP synthase, PPDK, and EI all carry out similar reactions, the transfer of a phospho group via a P-His intermediate between PEP and pyruvate.

Interaction between Acetate Kinase and EI

Acetate kinase catalyzes the conversion of acetate and ATP into acetyl phosphate and P_i. During the reaction a phospho group becomes linked to the enzyme via an acyl phosphate, a high-energy bond. It was shown that IIA^{Glc} could be phosphorylated by acetate kinase from *E. coli* or *S. typhimurium* in the presence of ATP (124). EI and HPr are required. Experiments with the purified enzymes showed that the phospho group of P-IIA^{Glc} can be transferred to acetate kinase if EI and HPr are present. It was shown that P-EI can directly donate its phospho group to acetate kinase, providing an explanation for these observations.

This reaction would establish a possible link between the PTS and the enzymes connected to the Krebs cycle (124). It should be pointed out, however, that this sequence of reactions has been shown only in an in vitro system with very high enzyme concentrations. It remains to be demonstrated that in an intact cell this alternate pathway to phosphorylate the PTS proteins in the absence of PEP is operative. Interestingly, lack of PEP has been suggested as the reason why *E. coli pfkA* mutants, lacking the major phosphofructokinase II, cannot grow on PTS carbohydrates that enter glycolysis at or above the level of fructose 6-phosphate (224, 397). Such *pfkA* mutants can grow on the corresponding carbohydrate phosphates, which are metabolized via 6-phosphogluconate. Kornberg and Smith (224) also showed that pyruvate could stimulate uptake of glucose, since it can generate PEP via PEP synthase. If phosphorylation of EI by phosphorylated acetate kinase can occur in vivo, acetyl phosphate might also stimulate the PTS, provided sufficient phosphorylated enzyme can be generated. It has been recently demonstrated that some of the chemotaxis proteins can be phosphorylated by phosphoramidate. CheY can also be phosphorylated by acetyl phosphate and carbamoyl phosphate (270) (see the next section).

Dannelly and Roseman have recently reported purification of a protein kinase that can phosphorylate EI on the active-site His residue (60). Although the activity of the EI-kinase was lost on purification, addition of $NAD(P)^+$ restored activity. Activity in crude extracts or partially purified fractions required no additional $NAD(P)^+$. The specific activity of the partially purified fraction is less than 50 pmol of EI phosphorylated per min per mg of protein, however, which is very low compared with the flux through the PTS.

Interaction between Carbon and Nitrogen Metabolism

Analysis of an ORF (ORF162) encoded downstream of the *K. pneumoniae rpoN* gene, which encodes a minor sigma factor, σ^{54}, showed that sequence similarity existed between ORF162 and the IIA^{Mtl} domains of *E. coli* and *Staphylococcus carnosus* and the IIA^{Fru} domains of *S. typhimurium* and *R. capsulatus* (384), especially around and including the active-site histidine. σ^{54} is required for the transcription of a number of genes important for nitrogen assimilation, especially under conditions of nitrogen limitation. It was speculated that ORF162 and related ORFs from *Pseudomonas putida* and *Bradyrhizobium japonicum* might be phosphorylated in a PTS-dependent manner. Since ORF162 might be a negative regulator of σ^{54}-dependent transcription (290), a link between carbon and nitrogen metabolism could be established in this way. However, no biochemical evidence exists at present that PTS-dependent phosphorylation of these ORFs occurs. Another possible link between nitrogen and carbon metabolism in the gram-positive organism *B. subtilis* is suggested by the finding that the *levR* gene product, involved in regulation of the levanase operon, has domains that are homologous to two families of bacterial activators (65). One domain is homologous to the antiterminators as discussed in the section on regulation through antiterminator proteins (above). The other domain is homologous to a part of the NifA and NtrC proteins, which are activators of σ^{54}-dependent transcription, possibly connecting it to regulation of nitrogen metabolism.

Poly(β-hydroxybutyric acid) Formation in *Alcaligenes eutrophus*

Alcaligenes eutrophus can accumulate poly(β-hydroxybutyric acid) (PHB). Mutants that accumulate less PHB, the so-called PHB-leaky mutants, have been isolated. A DNA fragment which complemented this phenotype was cloned and sequenced and shown to contain two genes which encode proteins that showed considerable similarity to EI and HPr from both gram-negative and gram-positive organisms (366). Possibly, a PHB-mobilizing system is phosphor-

ylated via EI/HPr and thus inactivated. Alternatively, transcription of genes involved in PHB accumulation might be regulated by the PTS in a way similar to that for the *bgl* and *sac* genes.

PTS AS A SIGNAL TRANSDUCTION SYSTEM IN CHEMOTAXIS

Many bacteria respond to environmental changes by swimming toward attractants and away from repellents. These behaviors are called positive and negative chemotaxis, respectively. Elaborate signal transduction networks of chemosensors and response regulators underlie this behavior (for a review, see references 323 and 470). One chemotaxis pathway involving methyl-accepting chemotaxis proteins (MCPs) as the membrane-bound sensors has been analyzed in great detail (for a review, see reference 30). MCPs bind but do not transport attractants (e.g., non-PTS carbohydrates or amino acids) and repellents via a periplasmic receptor domain. On stimulation, they transmit a signal through the cytoplasmic membrane to a cytoplasmic transmitter domain (or module) and the central processing unit.

Four general, cytoplasmic, chemotaxis proteins (CheA, CheY, CheW, and CheZ) are involved in signal transduction between the transmitter and the switch (proteins FliG, FliM, and FliN) of the flagellar motor. This switch eventually regulates the swimming behavior of the cells. CheA is an ATP-dependent protein kinase which in an unstimulated cell autophosphorylates slowly at a His residue. Positive MCP-dependent stimuli inhibit the rate of autophosphorylation, whereas negative stimuli increase autophosphorylation. Modulation of CheA activity involves sequestering of CheA together with CheW at the transmitter domain of MCPs. Activated CheA transfers its phospho group to an aspartate residue of the receiver and response regulator CheY. Phosphorylated CheY in turn locks the flagellar switch in clockwise rotation, thus causing tumbling of the cell. Dephosphorylation of CheY, most probably through CheZ, unlocks the switch. This allows the flagellar motor to return to counterclockwise rotation and the cell to swim smoothly (or run). Periodic alterations between run and tumble movements thus cause the random movement of a nonstimulated cell. Attractants prolong runs in the favorable direction by decreasing CheA autophosphorylation and cause positive chemotaxis. Repellents, in contrast, increase the phosphorylation of CheA, and subsequently of CheY, and thus cause a negative chemotaxis.

The PTS is also a signal transduction system through which bacteria respond by positive chemotaxis to the presence of PTS carbohydrates (for a review, see references 249 and 481). The activation of CheY during tumbling, a direct or indirect role of ATP in this process (241, 451), and an essential role for phosphorylation cascades in PTS-dependent chemotaxis (243) had been recognized some time ago in this system. In PTS-dependent chemotaxis, stimulation corresponds to uptake and phosphorylation of a substrate through an EII. No MCP is involved in this process, since mutants lacking the MCPs retained normal PTS-dependent chemotaxis (313, 331). Mutants lacking CheB and CheR, two proteins which are involved in the methylation-dependent adaptation of cells to MCP-dependent stimuli, also retained normal PTS-dependent chemotaxis. In *B. subtilis*, however, methyl transfer seems to be involved in chemotaxis toward PTS carbohydrates. This may indicate, similar to the MCP-dependent signals, variations in the chemotaxis mechanism in gram-positive bacteria compared with that in gram-negative bacteria (21).

For all EIIs analyzed thus far, transport/phosphorylation and chemoreception activities are closely correlated. Cells lacking a specific EII do not show chemotaxis toward the corresponding substrate, and the apparent affinities in wild-type cells for the three processes are very similar. In contrast, mutants lacking EI and HPr do not show a chemotactic response to any PTS carbohydrate, even if the corresponding EIIs are present (1, 237, 243, 289). Such unphosphorylated EIIs, as was shown directly through binding assays, bound the substrate with normal affinity but did not trigger a chemotactic response (147). In addition, mutant forms of II^{Mtl}, which cannot be phosphorylated but still bound the substrate, gave the same negative result (526). Extensive metabolism of the stimulating PTS carbohydrate is not necessary to elicit chemotaxis, however, since nonmetabolizable analogs are good attractants. Mutants defective in subsequent metabolism of PTS substrates also showed a normal chemotactic response (1, 237, 243). Furthermore, glucose 6-phosphate and fructose 6-phosphate did not cause chemotaxis in cells induced for an uptake system for hexose phosphates (Uhp). This result excludes the carbohydrate phosphates as the stimulating agents (331).

Despite an intensive search, no mutations in EIIs were found which eliminated chemotaxis but kept transport/phosphorylation intact (237, 243, 250, 481). Strains of *E. coli* carrying the genes encoding EIIs (EII^{Scr}, EII^{Sor}) from the nonmotile *K. pneumoniae*, or encoding intergenic EII hybrids, have also been tested. These hybrids consisted, for example, of $IICB^{Glc}$ from *E. coli* and IIA^{Nag} from *K. pneumoniae* or of $IIBC^{Scr}$ and IIA^{Nag}, both from *K. pneumoniae*. In all cases the heterologous and hybrid EIIs resulted in a chemotactic response and showed the specificity of the IIC substrate-binding domain (249, 461, 462). These results further corroborate the hypothesis that transport/phosphorylation and chemotactic stimulation in EIIs have steps in common.

Most sensory systems integrate various signals and adapt to long-lasting stimuli. Because all PTS-dependent phosphotransfer reactions merge at the general proteins EI and HPr, these proteins seemed logical candidates for this essential part of the signaling pathway. A model was proposed in which the signal for PTS-dependent chemotaxis is the change in the phosphorylation level of EI/HPr as a consequence of substrate transport (241, 243). The pleiotropic negative phenotype in chemotaxis of all *ptsH* and *ptsI* mutants tested thus far, and competition experiments, support the model. These experiments showed that factors which modulate the PTS phosphorylation activity (e.g., glucose 6-phosphate) similarly affected PTS-dependent chemotaxis (331).

Mutants which lacked HPr and which constitutively expressed *fruF* (and thus FPr) showed normal growth on and transport of the various PTS carbohydrates but lacked chemotactic activity (148). Even fructose did not cause a positive chemotaxis during uptake via the FPr-requiring fructose PTS but elicited a positive response during uptake via the HPr-requiring mannose PTS. Obviously, transport/phosphorylation and the chemotactic response are affected differently in these mutants. Similarly, mutants with a P11E mutation in HPr showed only a small decrease (about twofold) in transport activity but no chemotactic response. However, chemotaxis was restored to normal levels when the mutated HPr was overexpressed (148). These results

indicate that phosphorylation levels required for efficient transport are lower than those for efficient chemotaxis.

During stimulation by a PTS substrate, HPr and EI are presumably dephosphorylated. This could decrease, either directly or through a hypothetical intermediate phosphoryl chemotaxis protein, the level of P-CheA or P-CheY and result in smooth swimming and positive chemotaxis. Stimulation through the PTS could either decrease the activation of CheA (i.e., decrease the autophosphorylation of CheA), or increase the dephosphorylation of P-CheY by CheZ (249). Direct proof for this model is lacking thus far, and only circumstantial evidence for it can be given. In a strain lacking all MCPs, CheY and CheZ, and the general chemotaxis proteins CheA, CheB, CheR, and CheW, reintroduction of CheA, CheY, and CheW, but not of CheZ, is required to restore PTS-dependent chemotaxis (481). CheA, CheY and CheW are most probably needed to restore tumbling and thus a change in the direction of swimming of such cells. CheZ, however, could be dispensable because the dephosphorylated PTS stimulates dephosphorylation of P-CheY.

An essential role for IIA^{Glc}, adenylate cyclase, and cGMP has been postulated in linking the PTS to the general chemotaxis machinery (23). More recent studies showed, however, that the synthesis of the flagella, of the Che proteins, and of the EIIs was cAMP-CRP dependent, whereas PTS-dependent chemotaxis per se was normal in the absence of IIA^{Glc}, adenylate cyclase, cAMP, and the CRP protein. No evidence for a role of cGMP could be found (491, 508). In these studies *E. coli* strains carrying certain *cya* mutations were also tested. On addition of extracellular cAMP, some *cya* strains regained normal PTS-dependent transport/phosphorylation but not PTS-dependent chemotaxis. The allele in such mutants which uncouples PTS-dependent transport and chemotaxis was mapped in or close to the *crp* locus (508). It has been speculated that the mutation prevents, under the above conditions, the synthesis of an as yet hypothetical phosphoryl chemotaxis protein which normally couples the PTS with the general chemotaxis proteins (241).

Low-molecular-weight phospho-group donors such as acetylphosphate, carbamoylphosphate, and phosphoramidate also modulate the chemotactic response of enteric bacteria (537). This is perhaps through direct phosphorylation of CheY by these compounds in the absence of CheA (270). Whether this process and the reversible phosphorylation of EI and acetate kinase described previously (124) are relevant to understanding the missing link between the PTS and the central processing unit of the chemotaxis machinery remains to be shown.

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